الجامعة الأردنيـــــة
كلية الدراسات العليا

التاريـــخ: / /

نموذج رقم (16)
اقرار والتزام بالمعايير الأخلاقية والأمانة العلمية وقوانين الجامعة الأردنية وأنظمتها وتعليماتها لطلبة الدكتوراة

أنا الطالب: نسرين غازي محمد العبدللات   الرقم الجامعي: 9050079
تخصص: العلوم الحياتية /علم الدم   الكليـــــــة: العلوم

عنوان الاطروحة:

Antioxidant Properties of Selected Medicinal Plant Extracts on Human Blood

اعلن بأنني قد التزمت بقوانين الجامعة الأردنية وأنظمتها وتعليماتها وقراراتها السارية المفعول المتعلقة باعداد اطروحات الدكتوراه عندما قمت شخصيا" باعداد اطروحتي وذلك بما ينسجم مع الأمانة العلمية وكافة المعايير الأخلاقية المتعارف عليها في كتابة الأطروحات العلمية. كما أنني أعلن بأن اطروحتي هذه غير منقولة أو مستلة من أطاريح أو كتب أو أبحاث أو أي منشورات علمية تم نشرها أو تخزينها في أي وسيلة اعلامية، وتأسيسا" على ما تقدم فانني أتحمل المسؤولية بأنواعها كافة فيما لو تبين غير ذلك بما فيه حق مجلس العمداء في الجامعة الأردنية بالغاء قرار منحي الدرجة العلمية التي حصلت عليها وسحب شهادة التخرج مني بعد صدورها دون أن يكون لي أي حق في التظلم أو الاعتراض أو الطعن بأي صورة كانت في القرار الصادر عن مجلس العمداء بهذا الصدد.

توقيع الطالب:..........................   التاريخ:14 / 8 /2011

The University of Jordan

Authorization Form

I, Nesren Ghazi Al abdallat, authorize the University of Jordan to supply copies of my Thesis/ Dissertation to libraries or establishments or individuals on request, according to the University of Jordan regulations.

Signature:

Date: 15/ 8/ 2011

ANTIOXIDANT PROPERTIES OF SELECTED MEDICINAL PLANT EXTRACTS ON HUMAN BLOOD

By
Nesren Ghazi Al-abdallat

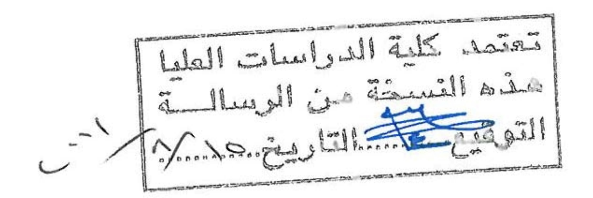
Supervisor
Dr. Yousif Y. Bilto, Prof

Co-Supervisor
Dr. Maher Salim, Prof

This Dissertation was Submitted in Partial Fulfillment of the Requirements for the Doctor of Philosophy Degree in the Biological Sciences/Hematology and Clinical chemistry

Faculty of Graduate Studies
University of Jordan

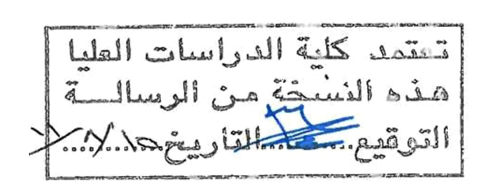
August, 2011



COMMITTEE DECISION

This Thesis/Dissertation (**Antioxidant Properties of Selected Medicinal Plant Extracts on Human Blood**) was Successfully Defended and Approved on: *Tuesday, July 5, 2011.*

Examination Committee	**Signature**
Dr. Yousif Y. Bilto, (Supervisor)	----------------------------
Prof. of Hematology	
Dr. Maher Salim, (Co-Supervisor)	----------------------------
Prof. of Biochemistry	
Dr. Abdullah AL-Abbadi (Member)	----------------------------
Prof. of Medicine /Hematology and Oncology	
Dr. Nabil Bashir (Member)	----------------------------
Prof. of Biochemistry and Molecular Biology	
(University of Science and Technology)	
Dr. Talal Aburjai (Member)	----------------------------
Prof. of Phytochemistry	
Dr. Shtaywy Abdalla (Member)	----------------------------
Prof. of Human Physiology	



This thesis is dedicated to………

…….my parents
my dear husband, Zaed and
my loving children, Ryhan, Abdulla,
Tima' and Joud

ACKNOWLEDGEMENTS

I would like to sincerely thank my supervisor Prof. Yousif Bilto for his very kind and generous support, academic advice, as well as his patience, understanding, expertise and criticism in the preparation of this manuscript. I would like to thank the other members of the advisory committee, Dr. M. Salim, Dr. Abdullah Abbadi, Dr. Nabil Bashir, Dr. Shtaywy Abdalla and Dr. Talal Aburjai for revision of the thesis, instructive comments and advice.

I also thank my parents for their support and understanding. A special thank you goes to my husband Zaed, and to my loving children, Ryhan, Abdulla, Tima'and Jod, for their help, encouragement, and patience during my program.

I would also to thank Dr. Maher Al-dabass for his useful suggestions during my work and Dr. Mosa Abuzurqa for the use of the rotary evaporator in his lab. Lastly, sincere thank you and gratitude to all of my friends for their support during my program.

LIST OF CONTENTS

LIST OF ABBREVIATIONS

ABBREVIATIONS………………………………………………………………...

AAE	Ascorbic Acid Equivalent
ABTS	2.2'- Azino-di[3-ethylbenzthiazoline sulphonate
COX-1, COX-2	Cyclooxygenases
CE	Catechol Equivalent
DPPH	2,2-diphenyl-1-picrylhydrazyl
DMSO	Di-Methyl Sulphoxide
DNPH	2, 4-dinitrophenylhydrazine
DTNB	5, 5'-Dithio-bis (2-nitrobenzoic acid)
Eo	Electron Reduction Potential
GPx	Glutathione peroxidase
GSH	Reduced glutathione
GSSG	Oxidized glutathione
GSTs	Glutathione S-transferases
Hb	Hemoglobin
H_2O_2	Hydrogen peroxide
IC_{50}	50% Inhibitory Concentration
I N T	2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyltetrazolium chloride
$L^{\cdot}$	Lipid radical
LDL-C	Low density lipoprotein-cholesterol
LOXs	Lipoxygenases
MDA	Malonyldialdehyde

mg/g Hb	milligram per gram Hemoglobin
mg/ml	milligram per milliliter
Mo	Molybdate
$NO^{\cdot}$	Nitric oxide
$NO_2^{\cdot}$	Nitric dioxide
nmol/g Hb	nanomole per gram Hemoglobin
$^1O_2$	Singlet oxygen
$O2^{\cdot -}$	Superoxide anion
$OH^{\cdot}$	Hydroxyl radical
$OONO^{-}$	Peroxynitrite
PBS	Phosphate Buffered Saline
PCV	Packed Cell Volume (hematocrite)
PC	Protein Carbonyl
PHGPX	Phospholipid Hydroperoxide Glutathione Peroxidase
PUFA	Polyunsaturated fatty acids
$R^{\cdot}$	Alkyl radicals
RE	Rutin Equivalent
$RO^{\cdot}$	Alkoxyl radical
$ROO^{\cdot}$	Peroxyl radical
ROOH	Alkyl peroxides
ROS	Reactive Oxygen Species
S.D.	Standard Deviation
TBA	Thiobarbituric acid

TCA	Tricloroacetic acid
XOD	Xanthine Oxidase

LIST OF TABLES

Table **Page**

LIST OF FIGURES

Figure **Page**

ANTIOXIDANT PROPERTIES OF SELECTED MEDICINAL PLANT EXTRACTS ON HUMAN BLOOD

By
Nesren Ghazi Al-abdallat

Supervisor
Dr. Yousif Y. Bilto, Prof

Co-Supervisor
Dr. Maher Salim, Prof

In the first part of this thesis, the antioxidant properties of 12 plant methanolic extracts were studied by chemical assays of total antioxidant capacity, DPPH free radical scavenging, ferric reducing and metal chelating activities. The extracts total phenols and flavonoids contents were also determined. The plant extracts were found to have different levels of antioxidant activities in the assays tested. The total antioxidant was expressed as µg/mg. Accordingly the studied plant extracts were arranged in the following decreasing order *Pistacia palaestina* (23.2%) > *Arbutus andrachne* (19.7%) > *Hypericum triquetrifolium* (18.6%) > *Zingiber officinale* (18.5%) > *Mentha Spp.* (18.4%) > *Rosmarinus officinalis* (18.3%) > *Salvia triloba* (15.4%) > *Verbena triphylla* (11.7%) > *Origanum syriacum* (10.9%) > *Teucrium polium* (9.6%) > *Nigella sativum* methanolic (8.0%) > *Ceratonia siliqua* (6.9%) > *Nigella sativum* ethanolic (5.7%) . The antioxidant activities of studied plant extracts were explained by their content of total phenols and flavonoids. *Nigella sativum* had the lowest antioxidant activities determined by the scavenging assays, despite of its highest metal chelating activity, indicating that it might exert its antioxidant activity in biological systems largely via its metal chelating property rather than free radical scavenging. This plant, however, could be used as a good source for new agents for iron chelating drugs.

In the second part of this thesis, the antioxidant effects of 12 plants on human erythrocytes exposed to H_2O_2 were studied in regard to lipid peroxidation (measured as MDA production), protein oxidation (measured as protein carbonyl production), erythrocyte reduced glutathione and hemolysis. The studied plant extracts were arranged in decreasing order of their *in vitro* anti-lipid-peroxidant activity in human erythrocytes as follows; *Zingiber officinale* >*Origanum syriacum* > *Rosmarinus officinalis* > *Arbutus andrachne* > *Pistacia palaestina* > *Hypericum triquetrifolium* > *Salvia triloba* > *Mentha Spp.* > *Verbena triphylla* > *Teucrium polium* > *Nigella sativum methanolic* > *Ceratonia siliqua* > *Nigella sativum ethanolic*. Whereas, the extracts were arranged in decreasing order of their *in vitro* anti-protein-oxidant activity in human erythrocytes as follows; *Hypericum triquetrifolium* >*Zingiber officinale* > *Nigella sativum* > *Rosmarinus officinalis* > *Teucrium polium* > *Salvia triloba* > *Verbena triphylla*. Unexpectedly *Arbutus andrachne*, *Pistacia palaestina*,

Mentha Spp. and Origanum syriacum although they were strong in free radical scavenging and iron chelating activities but had no anti-protein-oxidant activity. However, the following plant extracts: *Zingiber officinale*, *Origanum syriacum*, *Rosmarinus officinalis*, *Hypericum triquetrifolium*, *Arbutus andrachne*, *Pistacia palaestina*, *Salvia triloba*, *Mentha Spp*, *Verbena triphylla* and *Ceratonia siliqua* decreased significantly the percentage of hemolysis in erythrocytes exposed to 10 mM H_2O_2 that was explained by their anti-lipid-peroxidant activity. The tested plant extracts showed no significant effect on erythrocyte reduced glutathione.

I. Introduction:

Oxygen is a vital component for the survival of humans. It exists as a stable triplet biradical ($^3O_2$) in the ground state. Once inhaled, it undergoes a gradual reduction process and ultimately is metabolized to water. In this process, a small amount of reactive intermediates are produced. These reactive intermediates are collectively termed free radicals or reactive oxygen species (ROS) (Halliwell, 1995). Halliwell (1994) defined a free radical as any atom or molecule that contains one or more unpaired electrons, which causes them to become highly reactive chemical species. Hydroxyl radical ($OH^{\cdot}$), superoxide ($O_2^{\cdot -}$) and nitric oxide ($NO^{\cdot -}$) are the primary radicals formed and encountered by cells (Freeman and Crapo, 1982).

Cellular sources of free radicals range from enzymes located at the plasma membrane to intracellular enzymes and proteins which are present in the sub-cellular organelles and cytoplasm. These include plasma membrane NADPH oxidase (Mohazzab *et al.*, 1994), cytosolic xanthine oxidase (Fridovich, 1970; Berman and Martin, 1993), peroxisomal oxidases (Yeldandi *et al.*, 2000), cytochrome P-450 (Kuthan and Ullrich, 1982; Ekstrom and Ingelman-Sundberg, 1989; Puntarulo and Cederbaum, 1998; Serron *et al.*, 2000) and mitochondrial electron transport components (Turrens and Boveris, 1980). Environmental sources of free radicals are electromagnetic radiation, air pollutants and toxic chemicals (Freeman and Crapo, 1982).

Increased accumulation of free radicals may occur causing cellular injures; this is called oxidative stress. Oxidative stress is described as being an imbalance between free radicals production and their scavenging systems in cells, resulting in some oxidative

damage to biomolecules such as lipids, nucleic acids, proteins and carbohydrates (Sies *et al*., 2005). Oxidative damage increases the risk of several human chronic diseases, such as cancer, arteriosclerosis, neurodegenerative disorders, as well as aging processes (Aruoma, 1998). Furthermore, in biological systems, the high reactivity and very short lifetime of free radicals make their direct detection and identification difficult. Therefore the amount of damage resulted from free radical attack on these biomolecules are being used as an indirect detection of these radicals *in vivo* (Aruoma, 1998). One of the deleterious consequences of oxidative damage is lipid peroxidation, which involves hydrogen abstraction from fatty acids by free radicals such as $OH^{\cdot}$ and once initiated is a self – propagating process (Aruoma, 1998). Among the compounds that result from lipid peroxidation, malonyldialdehyde (MDA), which is widely used as an indicator of oxidative damage in clinical samples. Protein has also long been known to be susceptible to oxidation by free radicals. This oxidation may cause conversion of the side chain of some amino acid residues to carbonyl derivatives (Amici *et al*., 1989). Such alteration to protein subunits is a likely indicator of the increased protein oxidation with consequent degradation (Davies and Goldberg, 1987). On the other hand, recent studies suggest that the protein oxidation could also be responsible for the development of oxidative stress pathologies (Srour *et al*., 2000; Irhimeh, 2001). Nowadays, the most commonly measured products of protein oxidation in biological samples are the protein carbonyls, which are being used as indicators of oxidative injury, in addition to the above mentioned MDA.

Humans have antioxidant systems or defenses that protect them from free radicals. These antioxidant defense systems include enzymatic mechanisms such as glutathione-peroxidase (Gpx), superoxide dismutase (SOD) and catalase (CA). In addition, the human

body uses different antioxidant compounds contained in them such as, some large molecules (albumin, ceruloplasmin, ferritin, other proteins), and small molecules (ascorbic acid, glutathione, uric acid, bilirubin, tocopherol, carotenoids, (polyphenols) and some hormones (estrogen, angiotensin, melatonin, etc.) (Prior *et al.*, 2005).

Antioxidants have several definitions, but the common definition can be expressed as “any substance that delays or inhibits oxidation of oxidizable substrate by neutrilizing free radicals” (Antolovich *et al.*, 2002). Antioxidants are known to inhibit lipid peroxidation by either interfering with the chain reaction of lipid peroxidation, scavenging the free radicals or chelating free catalytic metals that mediate the production of free radicals (Halliwell and Gutteridge, 1984). Plants are rich in phenolic compounds and flavonoids which have been reported to exert multiple biological effects, such as antioxidant activities, free radical scavenging abilities, anti-inflammatory and anti-carcinogenic (Miller, 1996). The antioxidant activity of phenolics is mainly due to their redox properties, which allow them to act as reducing agents, hydrogen donors, and singlet oxygen quenchers. In addition, they have a metal chelation potential (Rice-Evans *et al.*, 1996). Epidemiological and *in vitro* studies on medicinal plants and vegetables strongly support the idea that plant constituents with antioxidant activity are capable of exerting protective effects against oxidative stress in biological systems (Cao 1996; Block and Patterson, 1992; Ness and Powles, 1997). Since crude extracts of herbs and other plant materials are rich in phenolics and flavonoids and since several studies reported a positive linear correlation between the total phenolic compounds and the antioxidant activities for aqueous and methanolic extracts of different plant species (Cai *et al.*, 2004; Tawaha *et al.*, 2007), the present study was directed towards the identification of antioxidant abilities of

methanolic extracts from the following parts of the plants: *Teucrium polium* (Aerial parts), *Nigella sativum* (seeds), *Zingiber officinale* (rhizomes), *Rosmarinus officinalis* (leaves), *Verbena triphylla* (leaves), *Mentha Spp.* (leaves), *Salvia triloba* (leaves), *Origanum syriacum* (leaves), *Hypericum triquetrifolium* (whole plant), *Ceratonia siliqua* (fruits), *Pistacia palaestina* (leaves) and *Arbutus andrachne* (leaves). The chemical and biological antioxidant properties of these plants were studied by *in vitro* experiments.

Previous studies showed that protein degradation induced by free radicals has more harmful effects on red blood cells than lipid peroxidation, particularely in regard to erythrocyte rheology (Srour *et al.,* 2000; Suboh *et al.,* 2004). These studies also showed that selected medicinal plants with known antioxidant properties may vary in protecting erythrocytes from oxidative stress, they may act as anti-lipid-peroxidant without preventing erythrocyte rheology loss or act as anti-protein-degradant with protection of erythrocyte rheology loss or act as having both activities. Therefore, in this proposed study, the antioxidant properties of the above mentioned plants were investigated to see whether they protect lipids and/or proteins in erythrocytes from oxidation. Furthermore, the possible mechanisms of action by which the antioxidant plants act against oxidative stress in erythrocytes will be assessed.

Aims of the present study were:

1. Finding out new potential plant species that have antioxidant activities. To determine these, a comprehensive screening study was carried out for the plant species mentioned above.

2. Extraction of plant parts mentioned above with a standard extraction procedures to get methanolic extract.
3. Evaluation of crude extraction yields for each plant material mentioned above in terms of g extract/ 100 g dry parts of plant.
4. Determining the antioxidant properties of plant extracts by chemical assays such as total antioxidant capacity, DPPH free radical scavenging activity, ferric reducing activity and metal chelating activity.
5. Determining the total phenol content and total flavonoids content in each plant to correlate them with the antioxidant activity.
6. Determining the antioxidant properties of plant extracts on human erythrocytes exposed to H_2O_2 by measuring erythrocyte MDA for lipid peroxidation, protein carbonyl for protein oxidation, erythrocyte reduced glutathione and oxidant hemolysis.

II. Literature review:

II.1. Medicinal plants in Jordan:

Jordan is well known for the great variation in wild plants that include not less than 2000 plant species belonging to about 700 genera. Among these plants, as many as 485 species from approximately 99 plant families are categorized as medicinal plants (Al-Eisawi, 1982; Oran, 1994; Oran and Al-Eisawi, 1998). Medicinal plants used in traditional medicine (Barakat and Fatma, 2003; T. Aburjai et al, 2007). Table I summarizes some medicinal plants included in this study according to their families, scientific, english and arabic names with traditional uses. The major constituents of studied plants were shown in table II.

Table I: List of plants included in this study according to their families, scientific, English and Arabic names with traditional uses.

Family	Scientific Name	English Name	Arabic Name	Part used	Traditional uses
Labiatae	*Mentha Spp.*	Mint	Na'Na'	Leaves	common cold, cough, influenza, constipation, male hypersexuality, nervosity
	Rosmarinusofficinalis L.	Rosemary	Ikleel al-Jabal or Iklil al-Jabal	Leaves	Obesity, constipation, kidney stones, hypertension,common cold, abdominal pains, ulcer, flatulence, toothache, edema, gynecological disorders, nervosity.
	Salvia triloba L.	Greek sage	Meriamia or Meirameieh	Leaves	Headache, flatulence, toothache, abdominal pain and common cold.
	Origanum syriacum L.	Thyme	Za'tar or Zaatar	Leaves	Blood coagulation, common cold, cough, influenza, abdominal pain, constipation.
	Verbena triphylla L.	Lemon verbena	Malleseh	Leaves	Abdominal pain, gynecological disorders, arthritis.
Leguminosae	*Ceratonia siliqua* L.	Carob Tree	Kharrob or Kharub	Fruits	Common cold, cough, influenza, indigestion, constipation nervosity.
Zingiberaceae	*Zingiber Officinale*	Ginger	Zanjabil	Rhizomes	Anemia, common cold, abdominal pains, indigestion, gynecological disorders, impotence, general weakness.
Ranunculaceae	*Nigella sativum* L.	blackseed	Habbat al-barakah	Seeds	Arthritis, general weakness, gynecological disorders, lactation deficiency, gastrointestinal problems, obesity, hypercholestrolemia, common cold, inflammations.
Ericaceae	*Arbutus andrachne* L.	Greek Strawberry Tree	Kaikab	Leaves	Urinary system, cancer, blood tonic, pectoral, antitussive, antiasthmatic.
Anacardiaceae	*Pistacia palaestina* Boiss	Turpentine Tree or Terebinth Tree	Butum	Leaves	Digestive system, urine intermittence diuretic, antihypertensive, antijaundice, diuretic, laxative, stimulant, aphrodisiac, astringent, anti-inflammatory, antiseptic, to heal burns and ulcers.
Lamiaceae	*Teucrium polium* L.	mountain germander	Jeada	Aerial parts	Antispasmodic, antiflatulence, antidiabetic, kidney stones.
Hypericaceae	*Hypericum triquetrifolium* Turra	Hypericum or St. John's wort	Roja	Whol plant	

Barakat E. Abu-Irmaileh, Fatma U. Afifi / Journal of Ethnopharmacology 89 (2003) 193–197 and T. Aburjai et al. / Journal of Ethnopharmacology 110 (2007) 294–304

Table II: major constituents of the studied plants

Scientific Name	Major constituents	refernce
Mentha Spp.	menthol , menthyl acetate, menthone and carvone	Marina et al., 2009
Rosmarinusofficinalis L.	hydrodistilled oil consist from 1,8-cineole, 2-ethyl-4,5-dimethylphenol, camphor, borneol, (+)-α-terpineol , α-pinene, and camphene.	Touafek, et al 2004
Salvia triloba L.	Twelve hydrocarbons, four sterols, three triterpenes, nineteen fatty acids, two phenolic acids, and five flavonoids have been detected and identified in Salvia triloba.	El-Sayed et al 2001
Origanum syriacum L.	carvacrol and/or thymol	Lukas et al., 2009
Verbena triphylla L.	polyphenols: verbascoside, various glycosyl- flavones (luteolin 7-glucoside, apigenin 7-galactoside, pedalitin 6-glucoside, apigenin 7-glucuronide, luteolin 7-diglucuronide , 6-hydroxyluteolin and 6- hydroxyapigenin or scutellarein glycosides.	Tomas-Barberan et al, 1988
Ceratonia siliqua L.	Carob fruits contain condensed tannins (proanthocyanidins), hydrolysable tannins (gallo- and ellagitannins).	Avallone et al, 1997
Zingiber Officinale	Paradols ,Gingerols and isogingerols , Shogaols and isoshogaol ,Gingerdiones , Methoxy-gingerols, Mono- and di-acetoxy-gingerdiols and Dihydro-[6]-paradols.	Jolad et al, 2005
Nigella sativum L.	Seed contained unsaturated fatty acids such as linoleic acid , and oleic acid , and saturated fatty acid such as palmitic acid. Myristic, myristoleic, palmitoleic, margaric, margaroleic, stearic, linolenic, arachidic, eicosenoic, behenic and lignoceric acids also present.	Cheikh-Rouhou et al., 2007
Arbutus andrachne L.	arbutin, ethyl gallate and catechin.	Fiorentino et al., 2007
Pistacia palaestina Boiss	The Essential oil is rich in monoterpenes, and the main	Flamini et al, 2004

	constituents are α-pinene and myrcene in the leaves and α-pinene, sabinene , and limonene in the galls. (*E*)-Ocimene, sabinene, and (*Z*)-ocimene are the main ones in both unripe and ripe fruits.	
Teucrium polium L.	Oil was rich in sesquiterpenes, carvacrol and Caryophyllene	Menichini, et al (2009)
Hypericum triquetrifolium Turra	Hypercidin and pseudohypercidin	Mulinnacci et al., 1999

II.2. Nature of Free Radicals:

Halliwell (1994) defined a free radical as any atom or molecule that contains one or more unpaired electrons, which causes them to become highly reactive chemical species. Hydroxyl radical ($OH^{\cdot}$), superoxide ($O_2^{\cdot -}$) and nitric oxide ($NO^{\cdot -}$) are the primary radicals formed and encountered by cells (Freeman and Crapo, 1982).

II.2.1. Reactive Oxygen Species (ROS) Classification:

ROS are constantly being generated in the body, as a result of the normal metabolic processes (Halliwell, 1999). Mitochondria, which consume more than 90% of the oxygen in aerobic living organisms, are the main source of ROS and free radicals. Approximately 1% to 5% of the oxygen consumed by mitochondria is reduced and converted to these ROS (Halliwell, 1991). ROS can be classified into oxygen-centered radicals such as superoxide anion ($O_2^{\cdot -}$), hydroxyl radical ($OH^{\cdot}$), alkoxyl radical ($RO^{\cdot}$), peroxyl radical ($ROO^{\cdot}$) and oxygen-centered non-radical derivatives such as hydrogen peroxide (H_2O_2) and singlet oxygen ($^1O_2$). Other common reactive species are nitrogen species such as nitric oxide ($NO^{\cdot -}$), nitric dioxide ($NO_2^{\cdot}$), and peroxynitrite ($OONO^-$) (Halliwell, 1994).

II.2.2. Reactive Oxygen Species (ROS) Sources:

Cellular sources of free radicals range from enzymes located at the plasma membrane to intracellular enzymes and proteins which are present in the sub-cellular organelles and cytoplasm. These include plasma membrane NADPH oxidase (Mohazzab *et al.*, 1994), cytosolic xanthine oxidase (Fridovich, 1970; Berman and Martin, 1993), peroxisomal oxidases (Yeldandi *et al.*, 2000), cytochrome P-450 (Kuthan and Ullrich, 1982; Ekstrom and Ingelman-Sundberg, 1989; Puntarulo and Cederbaum, 1998; Serron *et*

al., 2000) and mitochondrial electron transport components (Turrens and Boveris, 1980). Environmental sources of free radicals are electromagnetic radiation, air pollutants and toxic chemicals (Freeman and Crapo, 1982).

II. 2.3. Enzymatic Formation of ROS:

Lipoxygenases (LOXs) are found in plants and animals. LOXs are large monomeric proteins with non-heme iron cofactor containing dioxygenases [cyclooxygenases (COX-1, COX-2)] catalyzing the oxidation of the polyunsaturated fatty acids (PUFA) forming hydroperoxides (Sies and Cadenas, 1985; Sies, 1997). Substrates for LOXs in plants are linoleic acid (18:2) and linolenic acid (18:3) and in animals is arachidonic acid (20:4) (Brash, 1999). Because arachidonic acid either is not present in higher plants or is a minor constituent of cellular lipids, plant LOXs are classified into 9- and 13-LOXs with respect to their positional specificity of linoleic acid (Sies, 1997). In mammals, LOXs are classified into 5-, 8-, 12- and 15-LOX with respect to their positional specificity of arachidonic acid (Brash, 1999). In mammals, lipoxygenase needs free forms of PUFA, which are not present in healthy tissue. PUFA (e.g. arachidonic acid) can be released from glycerides by membrane bound phospholipase (Stief, 2000). Free PUFA are oxidized to form lipid hydroperoxides (Sies and Cadenas, 1985; Sies, 1997).

II. 3. Oxidative Stress, Human Diseases and Aging:

Oxidative stress is either due to overproduction of free radicals, or inadequate intake of the nutrients that contribute to the defense system. Oxidative stress has been associated with various human diseases. Yla-Herttuala *et al.* (1990) showed that a portion of the LDL extracted from the atherosclerotic lesion is similar to the oxidatively modified LDL. Antibodies against oxidised-LDL were generated in the atherosclerotic lesion but not in the normal artery (Palinski *et al.*, 1989). Steinberg (1997) later reported that the accumulation of cholesterol in the developing atherosclerotic lesion is not caused by the uptake of native LDL but the oxidised-LDL. Oxidised-LDL is internalised by macrophage receptors (Steinberg, 1997), leading to the generation of lipid peroxides that facilitates the accumulation of cholesterol esters (Ross, 1999). Besides atherosclerosis, lipid peroxidation has also been implicated in aging, cancer, multiple sclerosis, Parkinson's disease, Fanconi's anaemia and lupus (Harman, 1981; Halliwell and Gutteridge, 1984; Zhu *et al.*, 1994). Free radical oxidation is the most important biological reaction leading to pathological changes and aging of organisms (Harman, 1981). Protein alterations caused by oxidation are the main manifestation of aging (Yin and Chen, 2005). The levels of oxidised proteins increase with age in circulating erythrocytes. The change is correlated with decreases in the activities of marker enzymes, namely glyceraldehyde-3-phosphate dehydrogenase, aspartate aminotransferase, phosphoglycerate kinase and glucose-6-phosphate dehydrogenase. The oxidatively modified proteins were also found to be higher in the cultured human fibroblasts from donors above the age of 60 than from the young and middle-aged individuals (Oliver *et al.*, 1987). Similar changes were observed in the hepatocytes of old rats (Starke-Reed and Oliver, 1989). Furthermore, fibroblasts from

patients with progeria or Werner's syndrome, two genetic disorders associated with accelerated aging, containe higher levels of oxidised proteins than the age-matched controls (Oliver *et al.*, 1987). However, there is growing evidence that cardiovascular disease and cancer can be prevented or delayed by increased consumption of fruits, beverages, grains and vegetables. Thus, dietary antioxidants may augment the ability of the human body to counteract ill effects of free radicals (Halliwell, 1994).

II.3.1. Protein oxidation:

Proteins are highly susceptible to oxidative damage. Modification of proteins is mainly initiated by hydroxyl radicals, leading to the oxidation of amino acid side chains (where sulfur-containing amino acids are found) and protein fragmentation (Berlett and Stadtman, 1997; Stadtman and Levine, 2003). Malonaldehyde from lipid oxidation can react with protein amino groups to form adducts (Berlett and Stadtman, 1997; Virag *et al*., 2003). Peroxynitrite (ONOO–) can oxidize essential – SH groups on proteins (Virag *et al*., 2003). Protein oxidation alters signal transduction mechanisms, transport systems, and enzyme activities, and can lead to atherosclerosis and ischemia reperfusion injury (Stadtman, 2001). Aging is associated at least partly with oxidative modification of proteins (Berlett and Stadtman, 1997).

II.3.2. DNA strand breaks:

Mitochondrial DNA is susceptible to oxidative damage because of the lack of protective protein and close proximity to the reactive oxygen species-producing systems (Ames *et al*., 1993). Oxidative damage of DNA leads to mutagenesis and carcinogenesis (Ames *et al*., 1993). DNA can undergo oxidative damage at both the nucleic bases (the individual molecules that make up the genetic code) and at the sugars that link the bases

(Devasagayam *et al*., 1991). Oxidative damage of DNA results in degradation of the bases, breaking of the DNA strands by oxidation of the sugar linkages, or cross-linking of DNA to protein (a form of damage particularly difficult for the cell to repair). Although all cells have some capability of repairing oxidative damage to proteins and DNA, excess damage can cause mutations or cell death (Halliwell, 1992; Halliwell and Chirico, 1993).

II.3.3. Nucleic acid oxidation:

Nucleic acids are pentose-phosphate polymers that can undergo reactions with hydroxyl radical (Devasagayam *et al*., 1991). The base modifications in DNA nucleic acids may be responsible for genetic defects produced by oxidative stress. Among the four DNA bases, guanine has the lowest oxidation potential and is most easily oxidized (Devasagayam *et al*., 1991). Recently, 8-hydroxy guanosine has generated considerable interest as a biomarker of oxidative stress that can be used to estimate DNA damage in humans (Kasai, 1997). Urinary level of modified bases is a useful means of assessing the amount of DNA damage in an animal. Products such as 8-hydroxy guanosine, thymidine glycol, and uric acid are used for these estimates (Devasagayam *et al*., 1991). Formation of 8-oxoguanine induces Guanine: Cytidine to Thymine: Adenine transversions at the DNA replication stage, an important process in carcinogenesis and tumor development (Devasagayam *et al*., 1991). DNA damage has also been estimated by chain breaks and base modifications in cultured cells under oxidative stress. An important metabolic effect of DNA damage is the rapid induction of polyadenosine diphosphate ribose synthesis (ADP-ribosylation) in nuclei, resulting in extensive depletion of cellular NADH (Devasagayam *et al*., 1991). ADP-ribosylation has been associated with repair of damaged DNA.

II.3.4. Lipid Peroxidation in humans:

Lipid peroxidation is a self-propagation reaction that leads to generation of lipid radicals and lipid peroxides (Halliwell and Chirico, 1993). Cell membranes are phospholipid bilayers and proteins, which are the direct targets of lipid oxidation (Halliwell, 1992; Halliwell and Chirico, 1993). As lipid oxidation of cell membranes increases, the polarity of lipid-phase and formation of proteinoligomers increase but molecular mobility of lipids, number of SH groups, and resistance to thermodenaturation decrease (Halliwell, 1992; Halliwell and Chirico, 1993). During lipid oxidation, malonaldehyde (product of oxidation) can react with the free amino group of proteins, phospholipids, and nucleic acids damaging their structures and functions (Stadtman and Levine, 2003). Increased levels of lipid oxidation products are associated with diabetes and atherosclerosis (Halliwell, 1993; Halliwell and Chirico, 1993). Oxidation of low-density LDL has been reported to be involved in the development of atherosclerosis and cardiovascular disease (Halliwell, *et al*., 1992; Halliwell and Chirico, 1993). Oxidized cholesterol or fatty acid moieties in plasma LDL can lead to the development of atherosclerosis (Halliwell and Chirico, 1993). Lipid peroxidation (Figure 1) involves hydrogen abstraction to form a lipid radical ($L^{\cdot}$) (equation 1), which can react with oxygen to form a lipid peroxyl radical ($LOO^{\cdot}$) (equation 2). The peroxyl radical propagates the chain reaction by abstracting hydrogen from another lipid (equation 3), usually the rate-limiting step in lipid peroxidation (Shahidi and Wanasundara, 1992). Ideally, antioxidants work by blocking the propagation step (equation 3) through hydrogen atom donation.

Antioxidants can also interfere with the oxidation process by chelating catalytic metals and acting as free radical scavengers.

$$\mathbf{R^{\cdot} + LH \rightarrow RH + L^{\cdot}} \quad \text{(equation1)}$$

$$\mathbf{L^{\cdot} + O_2 \rightarrow LOO^{\cdot}} \quad \text{(equation 2)}$$

$$\mathbf{LOO^{\cdot} + LH \rightarrow LOOH + L^{\cdot}} \quad \text{(equation3)}$$

Figure 1: Lipid peroxidation steps (Shahidi and Wanasundara, 1992).

II.4. Antioxidant Defenses:

Antioxidants are known to inhibit lipid oxidation by interfering with the chain reaction of peroxidation or by scavenging the reactive oxygen radicals (Halliwell and Gutteridge, 1984). Survival of both prokaryotic and eukaryotic life forms is dependent on these substances which make up the biochemical defence system (Freeman and Crapo, 1982).The first line of defence is provided by superoxide dismutase (SOD), catalase and glutathione peroxidase, accompanied by metal binders and chelators such as transferrin, lactoferrin and a variety of siderophores. Radical scavengers, including α-tocopherol, β-carotene and ascorbate, are other forms of protection in the body. They do not prevent the oxidation process, but merely reduce its occurrence by allowing themselves to be consumed by free radicals (Machlin and Bendich, 1987). Mammalian plasma, bone marrow and intestinal mucosa have high antioxidant activity. In addition, β-globulin transferrrin and α-2-globulin caeruloplasmin are the major antioxidants in human plasma (Gutteridge and Stocks, 1981). Superoxide dismutase (SOD) which is found in the mitochondria and cytosol, converts superoxide to hydrogen peroxide (Halliwell, 1994). Catalase, present in the peroxisomes of most tissues, acts to remove peroxide generated by

peroxisomal oxidase enzymes, while glutathione peroxidase (GPX) removes hydrogen peroxide generated by SOD in the cytosol and mitochondria (Chance *et al.*, 1979). Tripeptide reduced glutathione (GSH) when oxidised by GPX formes glutathione disulphide (GSSG) (Sies and Summer, 1975; Nishiki *et al.*, 1976). Besides GPX, which acts predominantly in the aqueous phase, phospholipid hydroperoxide glutathione peroxidase (PHGPX) reduces hydroperoxides at the membranes (Maiorino *et al.*, 1991). Despite the presence of endogenous antioxidants, the body defence system is not efficient. Free radical production can lead to oxidative stress.

II.4.1. Enzymic Antioxidant Defenses in the body:

II.4.1.1. Superoxide dismutase (SOD):

SOD is present in cell cytoplasm (copper-zinc enzyme) and in mitochondria (manganese enzyme) in order to maintain a low concentration of superoxide anion (Halliwell, 1999). It catalyzes the dismutation of superoxide anion into oxygen and hydrogen peroxide according to the following reaction (equation 4):

$$\mathbf{2O_2^{\cdot -} + 2H^+ \xrightarrow{SOD} H_2O_2 + O_2} \qquad \text{(equation 4)}$$

II.4.1.2. Catalase:

Catalase is a heme protein that catalyses the detoxification of hydrogen peroxide (equation 5) (Halliwell, 1992). Catalase provides a protective role that is similar to that of glutathione peroxidase because both are important means of removing hydrogen peroxide (Harman, 2001).

Catalase

$$2H_2O_2 \longrightarrow 2H_2O + O_2 \qquad \text{(equation 5)}$$

II.4.1.3. Glutathione peroxidase (GPx):

Glutathione peroxidase (GPx) is a cytoplasmic and mitochondrial enzyme that is important for detoxifying peroxides in the cell (Halliwell, 1999). The GPx enzymes catalyze the reduction of H_2O_2 to water and organic peroxides (R-O-OH) to the corresponding stable alcohols (R-O-H) using glutathione (GSH) as areducing source (equation 6) (Halliwell, 1999).

$$ROOH + 2GSH \xrightarrow{GPx} ROH + GSSG + H_2O \qquad \text{(equation 6)}$$

II.4.1.4. Glutathione (GSH):

Glutathione (GSH, γ-glutamylcysteinylglycine), the primary non-protein sulfhydryl enzyme in aerobic organisms, is synthesized in most cells (Mezzetti *et al*., 1990). The tripeptide is formed by the combination of glutamic acid and cysteine, catalyzed by γ-glutamylcysteinyl synthetase. Glycine is then added by glutathione synthetase to form GSH (Mezzetti *et a*l., 1990). The sulfur atom in cysteine is able to partially ionize at neutral pH, i.e., the pH found in normal cells. This produces a different reactivity than the chemistry associated with a sulfhydryl group. Therefore in the case of glutathione there are two forms that differ only in the presence or absence of the proton on the cysteine moiety of the peptide (equation 7) (Mezzetti *et al*., 1990). The anionic form of glutathione is a strong nucleophile. Since there is very little of the anion at pH=7, cells have developed a family of enzymes called glutathione S-transferases that make glutathione a more reactive nucleophile (Halliwell, 1999).

$GSH \rightarrow GS^{-} + H^{+}$ (equation 7)

II.4.1.5. Glutathione S-transferases (GSTs):

GSTs are a large group of multifunctional proteins that catalyse the conjugation of GSH to various electrophilic substrates (Mezzetti *et al*., 1990). GSTs appear to play an important role in protecting cells against oxidative damage by: 1) binding glutathione in such a way that the sulfur is induced to ionize more completely, and 2) binding a second molecule close by so that a reaction can be facilitated (Halliwell, 1999). This reaction is necessary to detoxify xenobiotic materials such as toxins, drugs, and other foreign compounds (Mezzetti *et al*., 1990).

II.4.1.6. Glutathione reductase:

The flavoprotein, glutathione reductase, uses the reducing power for the pentose phosphate pathway (NADPH) to keep the glutathione pool in a cell in a very reduced state (equation 8) (Mezzetti *et al*., 1990). Cells contain at least 100 reduced glutathione molecules for every molecule of glutathione disulfide (Halliwell and Gutteridge, 1999).

$GSSG + NADPH \rightarrow 2GSH + NADP^{+} + H^{+}$ (equation 8)

The net result of this cycle is to use NADPH to reduce hydrogen peroxide to water, a process that requires two electrons (Halliwell and Gutteridge, 1999). Other reductases can also catalyze reactions that reduce lipid peroxides, i.e., LOOH, instead of hydrogen peroxide (equation 9).

$LOOH + 2GSH \rightarrow GSSG + LOH$ (equation9)

II.4.2. Non-enzymic Antioxidant Defenses in the body:

II.4.2.1. Tocopherols:

Tocopherols (i.e.Vit E) are natural constituents of biological membranes. Antioxidant mechanisms of tocopherols include the transfer of a hydrogen atom from the 6–hydroxyl group on the chroman ring, and scavenging of singlet oxygen and other reactive species (Noguchi and Niki, 1998). Tocopherols are regenerated in the presence of ascorbic acids (Noguchi and Niki, 1998). Tocopherols can protect PUFA within the membrane and LDL, and inhibit smooth muscle cell proliferation (Meydani, 2000). Tocopherol has been associated with the reduction of heart disease, delay of Alzheimer's disease, and prevention of cancer (Meydani, 2000). Tocopherols have beneficial effects in cardiovascular diseases by inhibiting LDL oxidation (Meydani, 2000).

II.4.2.2. Ascorbic acid:

The antioxidant mechanisms of ascorbic acid are based on hydrogen atom donation to lipid radicals, quenching of singlet oxygen, and removal of molecular oxygen (Liu and Meydani, 2002). Ascorbic acids help to regenerate tocopherols by donating a hydrogen atom to a tocopheroxyl radical. Ascorbic acid and tocopherol supplementation can substantially reduce oxidative damage (Liu and Meydani, 2002).

II.4.2.3. Natural Antioxidants in Plants:

II.4.2.3.1. Polyphenols and Flavonoids:

The primary constituents of phytochemicals that have the ability of contributing total antioxidant capacity of plants are the polyphenols (Lako *et al*. 2007). Polyphenols are widely present in plant kingdom and possessing significant bioactivities just like the antioxidant activity by adsorbing and neutralizing free radicals (Djeridane *et al*. 2006). Polyphenols can be classified as natural antioxidants that take an important place in our diet (Boskou *et al*. 2006). Polyphenols are among the most efficient antioxidant molecules owing to their ability to stabilize and delocalize the unpaired electron of free radicals by donating a hydrogen atom from their hydroxyl groups. There are many constituents of phenolics retaining potential antioxidant properties such as preventing agents against some critical diseases, independently or in synergetic action (Rice- Evans *et al*. 1997, Villano *et al*. 2004). Among the phenolic compounds, bioflavonoids are considered an important natural antioxidants that have an efficient free radical scavenging activity (Katalinic *et al*. 2006, Heim *et al*. 2002). the ability of such compound to act as free radical scavenger is partly related to its standard one-electron reduction potential (Eo). A lower Eo indicates that the chemical species is a better electron donor and thus a better reductant. Tea catechins have Eo values comparable to that of vitamin E, but higher than vitamin C (Jovanavic *et al.*, 1996 and 1997).

Polyphenols is a diverse class of plant secondary metabolites. They are characterised structurally by the presence of one or more six-carbon aromatic rings and two or more phenolic (i.e.., linked directly to the aromatic ring) hydroxyl groups (Figure 2). Flavonoids generally consist of two benzene rings (rings A and B) linked by an

oxygen-containing heterocycle (ring C) (Figure 3) (Lee *et al.*, 2004). There are six classes of flavonoids including: flavanones, flavones, flavonols (catechins and proanthocyanidins), isoflavonoids, anthocyanins, and flavans, which vary in structure. The antioxidant properties of flavonoids have been well studied and are structure-dependent with the major contributing factor being the catechol structure at ring B (two adjacent hydroxyl groups at 3′, 4′-positions). Hydroxyl substituents on the flavonoid ring increase the antioxidant activity, while substitution by methoxy groups decreases this activity (Manach et al., 2004).

Hydroxybenzoic acids

$R_1 = R_2 = OH$, $R_3 = H$: Protocatechuic acid
$R_1 = R_2 = R_3 = OH$: Gallic acid

Hydroxycinnamic acids

$R_1 = OH$: Coumaric acid
$R_1 = R_2 = OH$: Caffeic acid
$R_1 = OCH_3$, $R_2 = OH$: Ferulic acid

Chlorogenic acid

Flavonoids

See Figure 3

Stilbenes

Resveratrol

Lignans

Secoisolariciresinol

Figure 2: Structures of polyphenols, Manach et al., 2004.

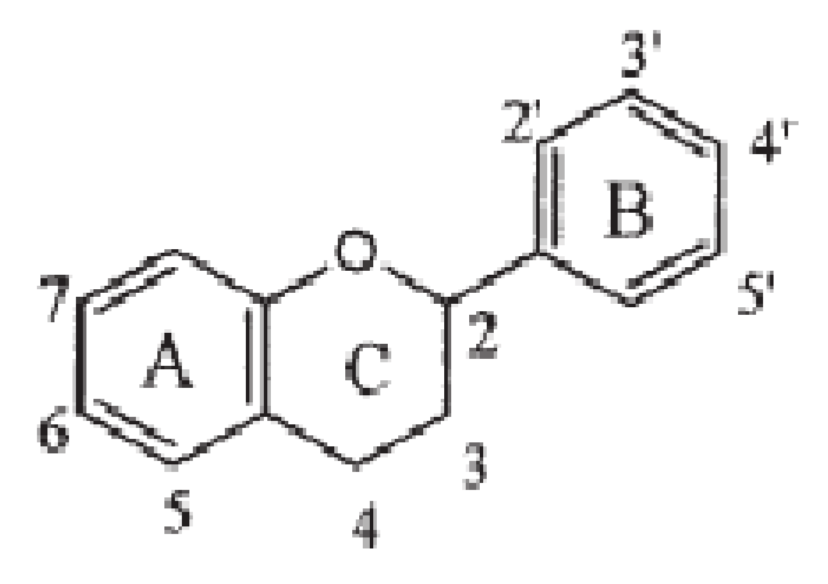


Figure 3: Structure of a flavonoid, Lee *et al.*, 2004.

III. Materials and methods:

III.1. Materials:

III.1.1. Plant material:

The following dried parts of plants: : *Pistacia palaestina* (leaves), *Hypericum triquetrifolium* (whole plant), *Arbutus andrachne* (leaves), *Mentha Spp.* (leaves), *Zingiber officinale* (rhizomes), *Rosmarinus officinalis* (leaves), *Salvia triloba* (leaves), *Verbena triphylla* (leaves), *Origanum syriacum* (leaves), *Tecrium polium (*arial parts), *Ceratonia siliqua* (fruits) and *Nigella sativum* (seeds) were used. *Hypericum triquetrifolium, Arbutus andrachne and Ceratonia siliqua* were collected from Mushager. *Pistacia palaestina* Boiss was collected from Ajloun Heights region. The others *Mentha Spp.*, *Zingiber officinale*, *Rosmarinus officinalis*, *Salvia triloba*, *Verbena triphylla*, *Origanum syriacum*, *Tecrium polium* and *Nigella sativum* were purchased from the local herbal stores in Madaba, Jordan. The choice of plant material used was dependent on reports of antioxidant activity and total phenolic content of selected Jordanian plant species (Tawaha *et al.*, 2007; Alali *et al.*, 2007) and the large use by public as folk medicine.

III.2. Methods:

III.2.1. Extraction method and yield:

All plant parts were dried under the same conditions (i.e. at room temperature, in a dark and clean place). The air dried plant parts were ground in a blender with a particular size to ensure the plant powders in identical size, and then 100 g of each plant powder were soaked for 5-7 days with 1000 ml of 98 % methanol at 25°C. After filtration, the

filtrate was evaporated with a rotary evaporator to remove the methanol under reduced pressure at 50°C. The dry crude extract for each plant was weighed out to determine extractive yield (g extract/100g dry plant) and then stored in refrigerator in glass bottles until use. An extra crude extract from *Nigella sativum* was also prepared using ethanol as solvent, in addition to the methanolic extract.

III.2.2. Antioxidant testing of plant extracts by chemical assays:

Antioxidant activities of the dry crude extracts of the plants were evaluated using *in vitro* chemical assays. For all chemical assays the same stock solution (1mg/ml) was prepared by dissolving 0.1g of dry crude extract in 100 ml of 98% methanol. Aliquots from this stock solution were further diluted with methanol as per the concentrations required.

III.2.2.1. Determination of total antioxidant capacity:

This assay is based on the reduction of Molybdinum (Mo) (VI) to Mo (V) by the extract and subsequent formation of a green phosphate/Mo (V) complex at acid pH (Prieto *et al*., 1999). The tubes containing 0.3 ml of extracts (1mg/ml) and 3 ml reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate) were incubated at 95° C for 90 min. After the mixture had cooled to room temperature, the absorbance of each solution was measured at 695 nm with single beam spectrophotometer (Novaspec 11, LKB Biochrome, England) against a blank. The blank tube contained 3.0 ml of reagent solution and the appropriate volume of the same solvent used for the sample (0.3 ml methanol/ethanol), and it is incubated under the same conditions as the sample. Total antioxidant capacity of plant extract was measured from the regression equation

(y=0.003x-0.035) prepared from the concentration versus optical density of ascorbic acid and the antioxidant capacity was expressed as ascorbic acid equivalent (AAE), (see Appendix I, Fig. 3).

III.2.2.2. Ferric reducing activity:

A method developed by Oyaizu (1986) was adopted for the determination of ferric reducing activity. One ml of plant extracts (1mg/ml) was mixed with 2.5 ml phosphate buffer (0.2 M, pH 6.6) and 2.5 ml of 1% potassium ferricyanide. The mixture was incubated at 50°C for 20 minutes, then rapidly cooled, mixed with 2.5 ml of 10% trichloroacetic acid and centrifuged at 3000 rpm for 10 minutes. 2.5 ml of supernatant was taken and then 2.5 ml of distilled water and 0.5 ml of 0.1% ferric chloride were added to it, mixed well and allowed to stand for 10 minutes. The absorbance was measured at 700 nm with single beam spectrophotometer (Novaspec 11, LKB Biochrome, England) against blank (Phosphate buffer at pH 6.6). Ascorbic acid was used as a reference standard. An increase in absorbance at 700 nm of the reaction mixture was interpreted as increase in ferric reducing activity of extract.

III.2.2.3. Metal chelating activity (Ferrous ion):

This method is based on the chelation of ferrous ions by the plant extracts (Dinis *et al*., 1994). Ferrozine can quantitatively form complexes with Fe^{2+}. In the presence of plant extract the complex formation is disrupted, resulting in a decrease in the red color of the complex and measurement of color reduction therefore allows estimation of the metal chelating activity of the coexisting chelators. Briefly, 1 ml of each extract at different

concentrations (10µg/ml - 200µg/ml) were mixed with 3.0 ml of distilled water, and then the mixture was mixed with 0.1 ml of 2mM $FeCl_2$ and allowed to stand for 20 min. The reaction was initiated by the addition of 0.2 ml of 5mM ferrozine solution. The absorbance of the solution was thereafter measured at 562 nm with single beam spectrophotometer (Novaspec 11, LKB Biochrome, England). Na_2EDTA was used as a positive control and the percentage inhibition of ferrozine–Fe^{2+}complex formation was calculated using the following formula:

$$\% \text{ Inhibition} = \frac{\text{Control absorbance} - \text{Test absorbance}}{\text{Control absorbance}} \times 100$$

III.2.2.4. Determination of DPPH radical-scavenging activity:

DPPH (1, 1-diphenyl-2-picrylhydrazyl) was used to determine the free radical scavenging activity of the extracts by the method of Bloiss (1958). DPPH radical was freshly prepared by dissolving 0.0024g DPPH in 100ml methanol (purple). To 1 ml of plant extract at different concentrations (10µg/ml - 200µg/ml), 1ml of DPPH was added and left in dark for 20 min., and then the absorbance was read at 517 nm with single beam spectrophotometer (Novaspec 11, LKB Biochrome, England). Ascorbic acid was used as a positive control. The percentage inhibition of DPPH formation was calculated using the following formula:

$$\% \text{ Inhibition} = \frac{\text{Control absorbance} - \text{Test absorbance}}{\text{Control absorbance}} \times 100$$

Calculation of 50% Inhibitory Concentration (IC_{50}):

IC_{50} value (the concentration of extract in µg/ml required to scavenge 50% of the DPPH free radical) was calculated from the inhibition curve using the percentage inhibition at five different concentrations of the extract.

III.2.2.5. Determination of total phenolic contents:

The amount of phenolic compounds present in the extracts was determined by Folin Ciocalteau reagent (Duh and Yen, 1997). Catechol (six concentrations ranging from 0.02 to 0.12 mg/ml) was used as a standard for the calibration curve. 1 ml of each plant extract (1mg/ml) was transferred into a 20 ml volumetric flask, and diluted with 10 ml of distilled water. 1 ml of 0.2 N Folin- Ciocalteau reagent (Sigma-Aldrich) was then added, followed by thorough mixing. After 3 minutes, 2 ml sodium carbonate (Na_2Co_3) at (100 g/l) was added, and the absorbance was measured at 760 nm with single beam spectrophotometer (Novaspec 11, LKB Biochrom, England) against blank. The total amount of phenolic compounds (mg/g) was determined from the calibration curve, and expressed as catechol equivalent (CE), (see Appendix I, Fig. 1).

III.2.2.6. Dertermination of total flavonoids content:

The amount of flavonoids in the exracts was determined by the Miliauskas method (2004) with slight modifications. Rutin (at six concentrations ranging from 0.05 to 0.5 mg/ml) was used as a standard for the calibration curve. One milliliter of each plant extract (1mg/ml) was mixed with 1 ml of 2 % aluminum trichloride ($AlCl_3$) in ethanol, after the

mixture was diluted with ethanol to 25 ml, allowed to stand for 40 minutes at 20°C, its absorbance was measured at 415 nm with single beam spectrophotometer (Novaspec 11, LKB Biochrom, England). In the blank, a mixture of 1 ml of the plant extract and 1 drop of acetic acid was diluted with ethanol to 25 ml, followed by measurement of the absorbance as described above. The total amount of flavonoids (mg/g) was determined from the calibration curve, and expressed as rutin equivalent (RE), (see Appendix I, Fig. 2).

III.2.3. Antioxidant testing of plant extracts by biological assays:

III.2.3.1. Antioxidant effects of plant extracts on human erythrocytes exposed to H_2O_2:

For each plant extract a stock solution (60 mg/ml) was prepared by dissolving 0.6 g of dry extracts in 1 ml of 100 % dimethylsulfoxide (DMSO) that was priorly diluted to 10 ml with phosphate buffered saline (PBS). Serial dilutions were prepared as follows (12 mg/ml, 24 mg/ml, 36 mg/ml and 48 mg/ml) (see Appendix I for PBS preparation). All solutions were stored in the refrigerator at 4 °C until use.

III.2.3.1.1. Blood Samples:

Apparently healthy university student volunteers of either sex, aging 19-30 years, have been chosen as a source of fresh blood after taking their consent. Venous blood from the antecubital vein was collected into lithium heparin tubes. Heparinized blood was used in all experiments.

III.2.3.1.2. Washing the erythrocytes:

Heparinized blood samples were centrifuged at 1200- 1500 xg for 5 min, then the plasma and buffy coat layer were removed (Dacie and Lewis, 1995 and Suboh, 2002). Packed erythrocytes were resuspended in cold phosphate buffered saline (PBS) and then centrifuged at 1200- 1500 xg for 5 min., this procedure was repeated three times. Washed erythrocytes were incubated with H_2O_2 with and without plant extracts and then used for malonyldialdehyde (MDA), carbonyl protein and reduced glutathione (GSH) measurements and percentage of oxidant hemolysis determination.

III.2.3.1.3. Packed Cell Volume (PCV):

The micro-haematocrit method is used for determination of PCV. Erythrocytes suspension was drawn into a plain capillary tube, until tube was 3/4 full. One end of the capillary was sealed and the tubes were centrifuged for 5 min at 1200 xg in the micro-haematocrit centrifuge. The percent PCV was calculated using a micro-haematocrit reader (Dacie and Lewis, 1995 and Suboh, 2002).

III.2.3.1.4. Hemoglobin determination:

The cyanmethemoglobin method (Dacie and Lewis, 1995 and Suboh, 2002) was used for hemoglobin determination. Twenty microliters of erythrocyte suspension were

mixed with 4.0 ml of Drabkin's reagent (see Appendix I for its preparation). The mixture was allowed to stand for 3-5 min at room temperature. The absorbance of the reaction mixture was read at 540 nm against reagent blank. Hemoglobin standard (stock: 14 g/dl) was diluted with Drabkin's reagent to give solutions corresponding to hemoglobin (Hb) concentrations of 3.5 to 10.5 g/dl, assayed as above and used for preparation of standard curve, (see Appendix I, Fig. 4).

III.2.3.1.5. Exposure of erythrocytes to H_2O_2:

Several preliminary experiments were carried out to determine the suitable H_2O_2 concentration to induce lipid peroxidation in washed erythrocytes, after which the 10 mM H_2O_2 concentration was reached.

Washed erythrocytes were suspended in phosphate buffered saline (PBS) with the PCV adjusted to 10% by hemoglobin estimation. Sodium azide was added to the suspending medium to 2.0 mM to inhibit catalas. These erythrocyte suspensions were pre-incubated for 1h at 37°C in shaking water bath. Next, equal volumes of erythrocyte suspension and H_2O_2 (20 mM) were mixed and incubated for another 60 min. at 37°C. Control tubes contained PBS instead of H_2O_2. After the incubation period, the suspensions were used for erythrocytes MDA, protein carbonyl, reduced glutathione (GSH) and percentage of oxidant hemolysis determinations (Srour, 1998; Srour *et al.*, 2000; Suboh, 2002 and Suboh *et al*., 2004). A given plant extract was added to erythrocyte suspensions during the pre-incubation period with sodium azide. The final concentrations of plant extracts were as follows (0.2 mg/ml, 0.4 mg/ml, 0.6 mg/ml and 0.8 mg/ml). After 30 min of incubation with plant extracts at 37°C in shaking water bath, equal volume of H_2O_2 (20

mM) was added. Further incubation for 60 min under the same condition was performed to determine their antioxidant activities.

III.2.3.1.6. Erythrocyte MDA measurement:

Erythrocyte MDA was determined by the Stocks and Dormandy (1971) method with slight modifications (Srour *et al*., 2000). Briefly, to 3.0 ml of erythrocyte suspensions (PCV=5%), 2.0 ml of Trichloro Acetic acide-Arsenate (TCA 28%, Arsenate 0.1 M) was added and mixed. This mixture was centrifuged for 15 min at 1050 xg. An aliquot (2.0 ml) of the supernatant was mixed with 0.5 ml of Thiobarbituric acid (TBA) solution (1% in 0.05 M NaOH). This mixture was placed in boiling water bath for exactly 15 min, then immediately cooled under tap water. The absorption of the reaction mixture was read at 532 nm against blank. Standard MDA (1, 1, 3, 3,-tetraethoxypropane) solutions (4-20 nmol/ml) was assayed as above to construct a standard curve (see Appendix I, Fig. 6). All MDA concentrations were expressed in nmol/g Hb.

III.2.3.1.7. Protein Carbonyl measurement in erythrocyte:

Cayman's protein carbonyl assay kit was used (Reznick and Packer, 1994). This assay is based on the reaction between 2, 4-dinitrophenylhydrazine (DNPH) and protein carbonyls in hemolysates of cell suspensions (PCV=5%) in a convenient 96-well format. DNPH reacts with protein carbonyls, forming protein-hydrozone, which can be quantified spectrophotometrically at an absorbance of 405 nm. All protein carbonyl concentrations were expressed in nmol/g Hb.

Procedure:

1. Transfer 1.0 ml of cell suspensions (PCV= 5%) to two 2 ml plastic tubes. One tube will be the sample tube and the other will be the control tube.

2. Add 2ml of DNPH (20 mM) to the sample tube and add 2ml of 2.5 M HCl to the control tube. Incubate both tubes in the dark at room temperature for two hours. Vortex each tube briefly every 15 minutes during the incubation.

3. Add 1 ml of 20% TCA to each tube and vortex, then centrifuge tubes at 10,000 xg for 10 minutes.

4. Discard the supernatant and resuspend the pellet in 1 ml of (1:1) ethanol/ethyl acetate mixture.

5. Manually suspend pellet with spatula, vortex thoroughly, and centrifuge tubes at 10,000 xg for 10 minutes in a centrifuge. Repeat this step twice.

6. After the final wash, resuspend the protein pellets in 500 µl of guanidine hydrochloride by vortexing and then centrifuge tubes at 10,000 xg for 10 minutes to remove any leftover debris.

7. Transfer 220 µl of supernatant from the sample tubes and control tubes to the wells of the 96-well plate.

8. Measure the absorbance at a wavelength at 405 nm using a plate reader.

III.2.3.1.8. Erythrocyte Reduced Glutathione (GSH) Determination:

Reduced glutathione was determined using the modified method of Ellman (1951). Briefly, to 1ml hemolysate of erythrocyte suspension (PCV=5%), 2 ml of precipitating solution (see Appendix 1 for its preparation) was added, mixed and allowd to stand for 5

min at room temperature. Then, the mixture was centrifuged at 4200 xg for 10 min. To 1.0 ml of the supernatant, 2 ml of phosphate solution (0.3 M of Na_2HPO_4) and 0.5 ml of 5, 5'-Dithio-bis (2-nitrobenzoic acid) (DTNB) (Ellman's reagent; 40 mg/dl) were added. The assay mixture was mixed and its absorbance was read within 4 min at 412 nm against blank. Standard glutathione (reduced) solutions (5-20 mg/dl) was assayed as above to construct a standard curve (see Appendix I, Fig. 5). All glutathione (GSH) concentrations were expressed in mg/g Hb.

III.2.3.1.9. Percentage of oxidant hemolysis:

Percentage of oxidant hemolysis was determined by diluting and mixing 0.1 ml of the cell suspensions (PCV=5%) with 2.9 ml distilled water, to induce complete hemolysis. All samples before and after dilution with water were then centrifuged at 1200 xg for 5 min. and hemoglobin concentration of supernatants was determined spectrophotometrically at 540 nm. Percentage hemolysis was calculated from the ratio of the absorbance of pre- to post- diluted samples (Davies and Goldberg, 1987 and Suboh, 2002).

III.3. Statistical Analysis:

All data were reported as the mean ± standard deviation.The results with a value $P < 0.05$ were considered significant. IC_{50} was calculated by using microsoft excel.

IV. Results:

IV.1. Extractive yield:

Extractive yield (g extract/ 100g dry plant) for each plant was determined and the largest extraction yield by methanol was obtained for *Arbutus andrachne* (31.0%), followed by *Pistacia palaestina* (30.5%), *Hypericum triquetrifolium* (27%), *Ceratonia siliqua* (23.2%), *Nigella sativum* (ethanolic) (20%), *Salvia triloba* (15.5%), *Nigella sativum* (methanolic) (13.4%), *Rosmarinus officinalis* (12%), *Origanum syriacum* (7.1%), *Teucrium polium* (6.2%), *Verbena triphylla* (4.1%), *Mentha Spp.* (3.9%).

IV.2. Antioxidant testing of plant extracts by chemical assays:

IV.2.1. Total antioxidant capacity:

Figure 4 shows the total antioxidant capacities (µg ascorbic acid equivalent/mg extract) of plant extracts in decreasing order. As shown in Fig. 4 the highest activity was found in, *Pistacia palaestina* (232 µg/mg), followed by *Arbutus andrachne* (197 µg/mg), *Hypericum triquetrifolium* (186 µg/mg), *Zingiber officinale* (185 µg/mg), *Mentha Spp.*(184 µg/mg), *Rosmarinus officinalis* (183 µg/mg), *Salvia triloba* (154 µg/mg), *Verbena triphylla* (117 µg/mg), *Origanum syriacum* (109 µg/mg), *Teucrium polium* (96 µg/mg), *Nigella sativum* (methanolic extract) (80 µg/mg), *Ceratonia siliqua* (69 µg/mg) with *Nigella sativum* (ethanolic extract) (57 µg/mg) being the lowest.

IV.2.2. DPPH free radical scavenging activity:

The IC_{50} values for the DPPH free radical scavenging activities of the extracts are shown in Figure 5 in decreasing order compared to ascorbic acid as a positive control (raw data are shown in Appendix 1). As shown in Fig. 5, compared to ascorbic acid (IC_{50}=5.8 µg/ ml), the highest scavenging activity for DPPH was observed in *Pistacia palaestina* (IC_{50}= 14.9 µg/ml), followed by *Arbutus andrachne* (IC_{50}= 15.2 µg/ml), *Hypericum triquetrifolium* (IC_{50}= 23.5 µg/ml), *Salvia triloba* (IC_{50}= 24.8 µg/ml), *Mentha Spp*. (IC_{50}= 26.2 µg/ml), *Rosmarinus officinalis* (IC_{50}= 30.4 µg/ml), *Verbena triphylla* (IC_{50}= 32.4 µg/ml), *Zingiber officinale* (IC_{50}= 40.4 µg/ml), *Origanum syriacum* (IC_{50}= 55.8 µg/ml), *Teucrium polium* (IC_{50}= 79.0 µg/ml), *Nigella sativum* (methanolic extract) (IC_{50}= 3013 µg/ml), *Nigella sativum* (ethanolic extract) (IC_{50}= 4043 µg/ml) with *Ceratonia siliqua* IC_{50}= (5350 µg/ml) being the lowest.

IV.2.3. Ferric reducing activity:

Figure 6 shows the ferric reducing activities of plant extracts in decreasing order compared to ascorbic acid. As shown in Fig. 6, compared to ascorbic acid (O.D=3), the highest activity was observed in *Pistacia palaestina* (O.D=2.7), followed by *Hypericum triquetrifolium* (O.D=2.1), *Arbutus andrachne* (O.D=2.0), *Zingiber officinale* (O.D=1.4), *Mentha Spp*.(O.D=1.36), *Rosmarinus officinalis* (O.D=1.35), *Salvia triloba* (O.D=1.2), *Verbena triphylla* (O.D=1.0), *Origanum syriacum* (O.D=0.83), *Teucrium polium* (O.D=0.34), *Nigella sativum* (methanolic extract) (O.D=0.25), *Ceratonia siliqua* (O.D=0.20) with *Nigella sativum* (ethanolic extract) (O.D=0.13) being the lowest.

IV.2.4. Metal chelating activity:

Figure 7 shows the IC_{50} values for the ferrous chelating activities of plant extracts in decreasing order compared to EDTA as a positive control (raw data are shown in Appendix 1). As shown in Fig. 7, compared to EDTA (IC_{50}= 17.3µg/ml), the highest chelating activity was observed in *Nigella sativum/* methanolic extract (IC_{50}= 75 µg/ml), followed by *Nigella sativum/* ethanolic extract (IC_{50}= 77 µg/ml), *Pistacia palaestina* (IC_{50}=87.7 µg/ml), *Arbutus andrachne* (IC_{50}= 96.3 µg/ml), *Hypericum triquetrifolium* (IC_{50}= 162.1 µg/ml), *Teucrium polium* (IC_{50}= 304 µg/ml), *Mentha Spp*.(IC_{50}= 896 µg/ml), *Origanum syriacum* (IC_{50}=1002 µg/ml), *Verbena triphylla* (IC_{50}=1601 µg/ml), *Zingiber officinale* (IC_{50}=2095 µg/ml), *Salvia triloba* (IC_{50}=5019 µg/ml) and *Rosmarinus officinalis* (IC_{50}=12087 µg/ml) being the lowest. *Ceratonia siliqua*, however, did not show any metal chelating activity at all.

IV.2.5. Total phenolic and flavonoid contents:

The total phenolic contents (mg catechol equivalent/g extract) of the plant extracts are shown in Fig. 8 in decreasing order. As shown in Fig. 8, the highest amount of phenols was observed in *Pistacia palaestina* (149 mg/g), followed by *Hypericum triquetrifolium* (124 mg/g), *Arbutus andrachne* (105 mg/g), *Zingiber officinale* (91 mg/g), *Mentha Spp*.(71 mg/g), *Rosmarinus officinalis* (66 mg/g), *Salvia triloba* (64 mg/g), *Verbena triphylla* (52 mg/g), *Origanum syriacum* (48 mg/g), *Teucrium polium* (28 mg/g), *Nigella sativum/*methanol (25 mg/g), *Ceratonia siliqua* (23mg/g) and with *Nigella sativum/*ethanol (16 mg/g) being the lowest.

The total flavonoid contents (mg rutin equivalent/g extract) of the plant extracts are shown in Fig. 9 in decreasing order. As shown in Fig. 9, the highest amount of flavonoids was observed in *Hypericum triquetrifolium* (171 mg/g), followed by *Pistacia palaestina* (115 mg/g), *Arbutus andrachne* (112 mg/g), *Mentha Spp.* (104 mg/g), *Zingiber officinale* (70 mg/g), *Rosmarinus officinalis* (67 mg/g), *Salvia triloba* (66 mg/g), *Verbena triphylla* (65 mg/g), *Origanum syriacum* (59 mg/g), *Teucrium polium* (56 mg/g), *Nigella sativum/*methanol (53mg/g), *Ceratonia siliqua* (43 mg/g) and *Nigella sativum/*ethanol (29 mg/g) being the lowest.

IV.2.6. Correlation of chemical screening results of plant extracts:

Table II, shows the correlation between the results of chemical screening for the plant extracts and the percentages of total antioxidant capacity (TAC) of the extracts and the dry plants.

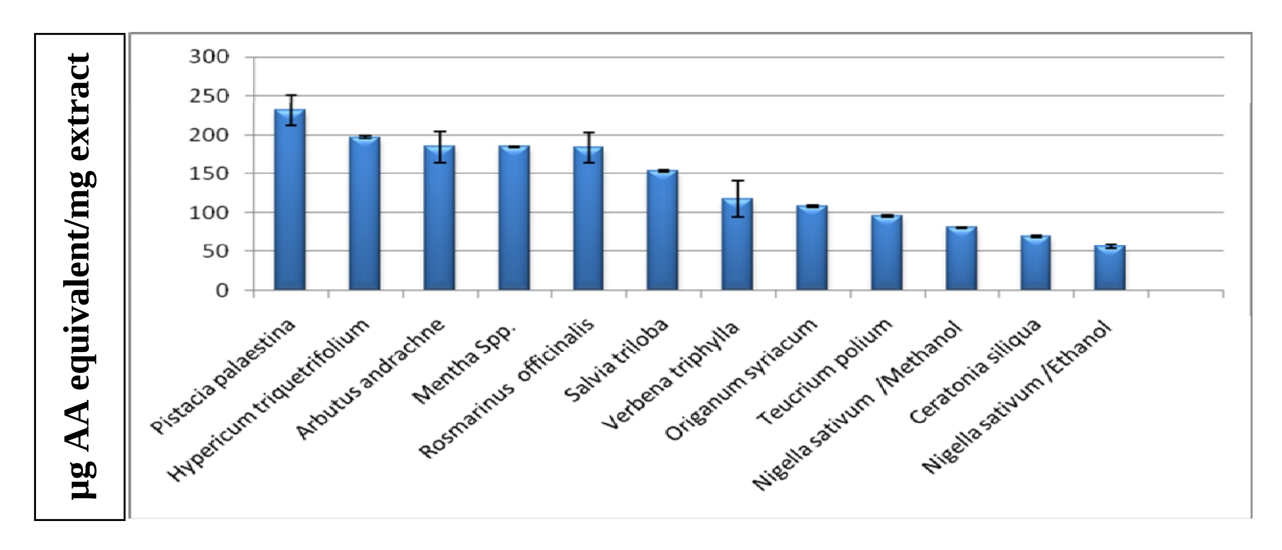


Figure (4): Total antioxidant capacity (µg AA equivalent/mg extract) of *Pistacia palaestina*, *Arbutus andrachne*, *Hypericum triquetrifolium*, *Zingiber officinale*, *Mentha Spp.*, *Rosmarinus officinalis*, *Salvia triloba*, *Verbena triphylla*, *Origanum syriacum*, *Teucrium polium*, *Nigella sativum* (methanol), *Ceratonia siliqua* and *Nigella sativum* (ethanol) extracts. Each column represents the mean value and bars represent the S.D. (n = 6).

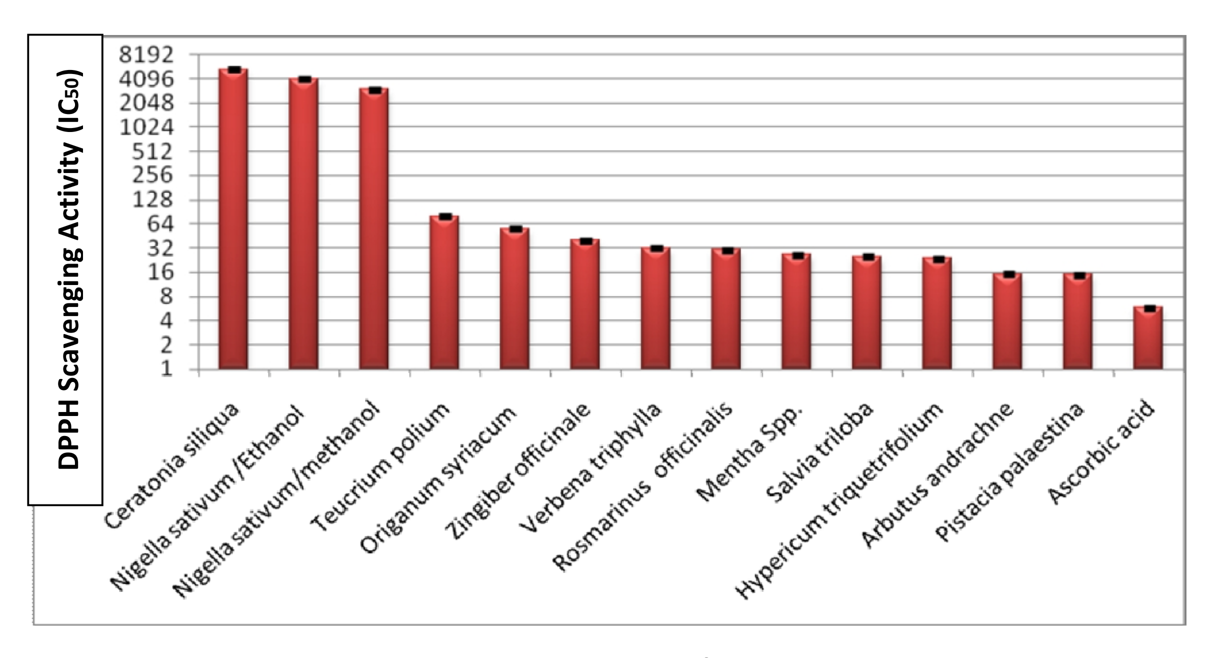


Figure (5): IC_{50} (µg/ml) DPPH Scavenging Activity of ascorbic acid, *Pistacia palaestina*, *Arbutus andrachne*, *Hypericum triquetrifolium*, *Salvia triloba*, *Mentha Spp.*, *Rosmarinus officinalis*, *Verbena triphylla*, *Zingiber officinale*, *Origanum syriacum*, *Teucrium polium*, *Nigella sativum* (methanol), *Nigella sativum* (ethanol) and *Ceratonia siliqua* extracts. Each column represents the mean value and bars represent the S.D. (n = 3).

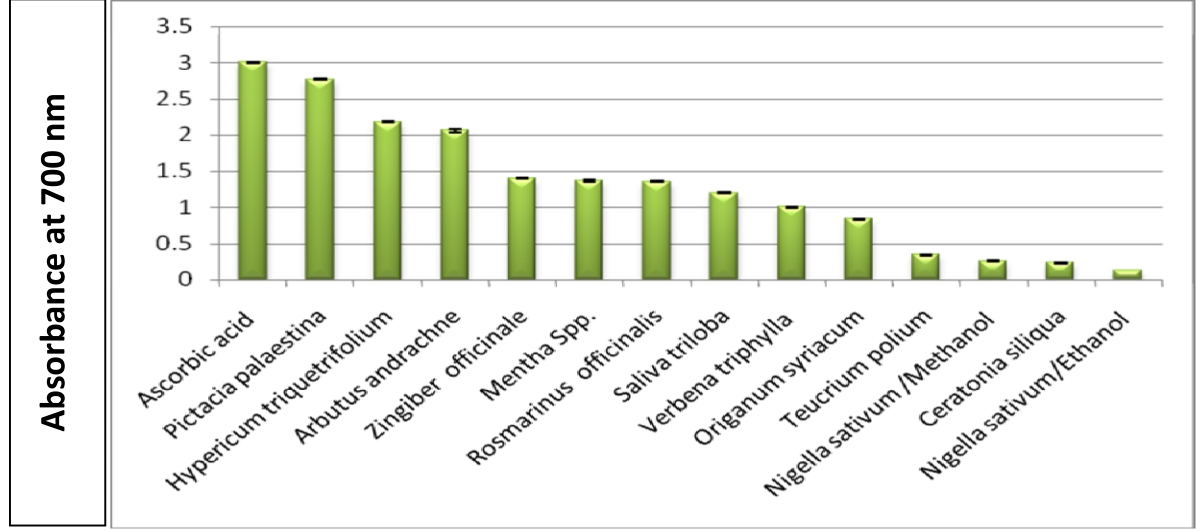


Figure (6): Reducing power (Absorbance at 700 nm) of Ascorbic acid, *Pistacia palaestina*, *Hypericum triquetrifolium*, *Arbutus andrachne*, *Zingiber officinale*, *Mentha Spp.*, *Rosmarinus officinalis*, *Salvia triloba*, *Verbena triphylla*, *Origanum syriacum*, *Teucrium polium*, *Nigella sativum* (methanol), *Ceratonia siliqua* and *Nigella sativum* (ethanol) extracts. Each column represents the mean value and bars represent the S.D. (n = 6).

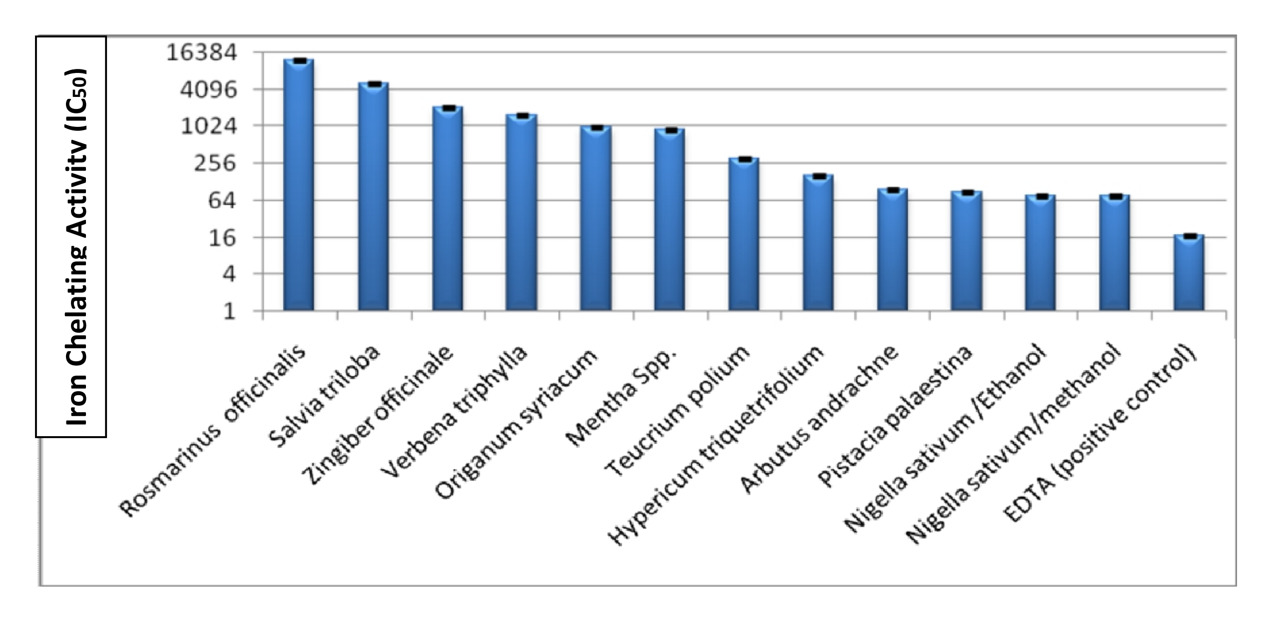


Figure (7) : IC_{50} (µg/ml) Iron Chelating Activity of EDTA, *Nigella sativum* (methanol), *Nigella sativum* (ethanol), *Pistacia palaestina*, *Arbutus andrachne*, *Hypericum triquetrifolium*, *Teucrium polium*, *Mentha Spp.*, *Origanum syriacum*, *Verbena triphylla*, *Zingiber officinale*, *Salvia triloba* and *Rosmarinus officinalis* extracts, Each column represents the mean value and bars represent the S.D. (n = 3).

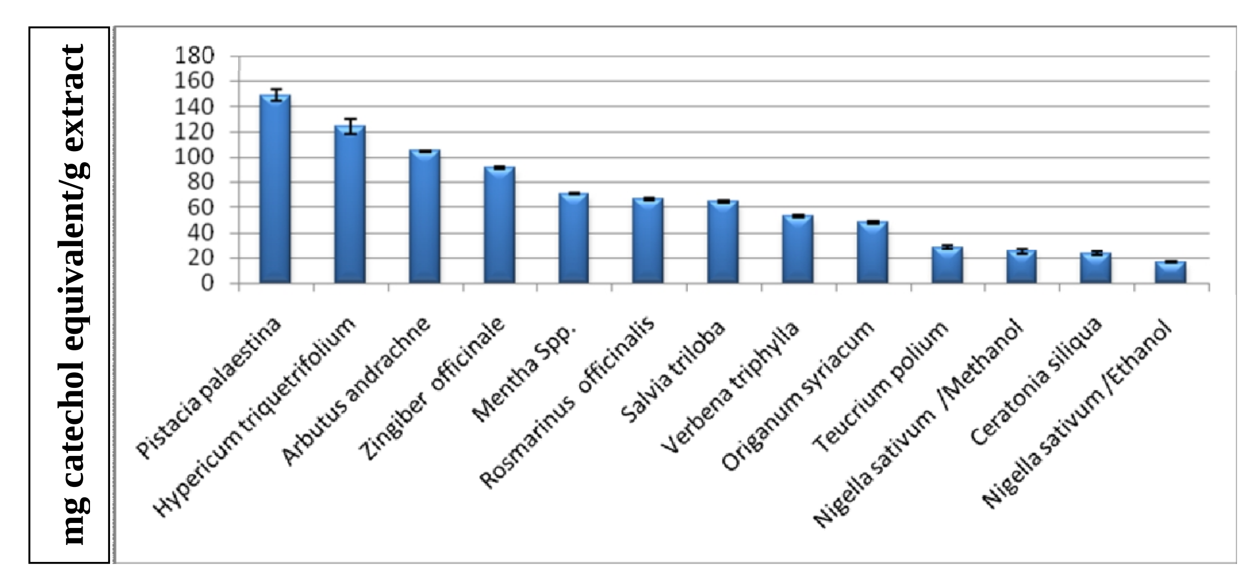


Figure (8): Total phenolic content (mg catechol equivalent/g extract) of *Pistacia palaestina*, *Hypericum triquetrifolium*, *Arbutus andrachne*, *Zingiber officinale*, *Mentha Spp.*, *Rosmarinus officinalis*, *Salvia triloba*, *Verbena triphylla*, *Origanum syriacum*, *Teucrium polium*, *Nigella sativum* (methanol), *Ceratonia siliqua* and *Nigella sativum* (ethanol) extracts. Each column represents the mean value and bars represent the S.D., (n = 6).

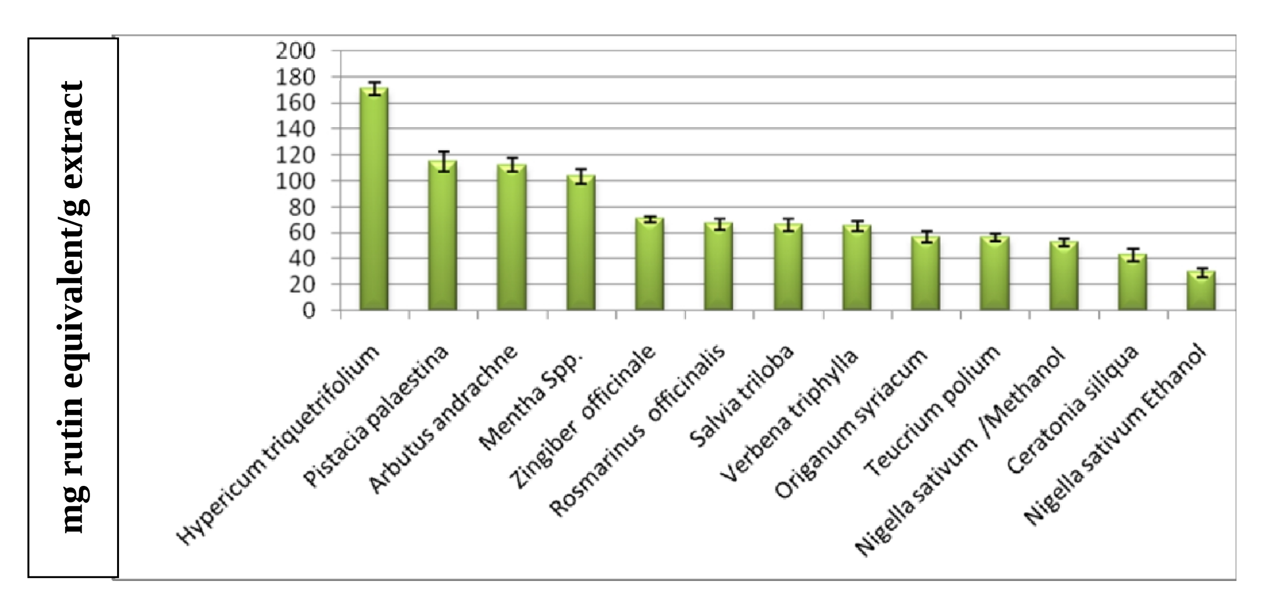


Figure (9): Total flavonoids content (mg rutin equivalent/g extracrs) of *Hypericum triquetrifolium*, *Pistacia palaestina*, *Arbutus andrachne*, *Mentha Spp.*, *Zingiber officinale*, *Rosmarinus officinalis*, *Salvia triloba*, *Verbena triphylla*, *Origanum syriacum*, *Teucrium polium*, *Nigella sativum* (methanol), *Ceratonia siliqua* and *Nigella sativum* (ethanol) extracts. Each column represents the mean value and bars represent the S.D. (n = 6).

Table III:Correlation of chemical screening results of plant extracts and percentages of TAC for the extracts and the dry plants

Plant	% yield (g/100g)	phenols mg/g	Flavonoids mg/g	TAC µg/mg	DPPH IC_{50} (µg/ml)	Ferric reducing O.D	Metal chelating IC_{50} (µg/ml)	TAC % extract (g/100g)	TAC % dry plant (g/100g)
Pistacia palaestina	**30.5%**	**149**	**115**	**232**	**9.5**	**2.7**	**119**	**23.20%**	**7.10%**
Arbutus andrachne	**31%**	**105**	**112**	**197**	**19**	**2**	**124**	**19.70%**	**6.10%**
Hypericum triquetrifolium	**27%**	**124**	**171**	**186**	**18**	**2.1**	**154**	**18.60%**	**5.00%**
Zingiber officinale	**7.7%**	**91**	**70**	**185**	**21**	**1.4**	**212**	**18.50%**	**1.40%**
Mentha Spp.	**3.9%**	**71**	**104**	**184**	**30**	**1.36**	**156**	**18.40%**	**0.70%**
Rosmarinus officinalis	**12%**	**66**	**67**	**183**	**41**	**1.35**	**241**	**18.30%**	**2.20%**
Salvia triloba	**15.5%**	**64**	**66**	**154**	**46**	**1.2**	**211**	**15.40%**	**2.40%**
Verbena triphylla	**4.1%**	**52**	**65**	**117**	**64**	**1**	**233**	**11.70%**	**0.50%**
Origanum syriacum	**7.1%**	**48**	**59**	**109**	**90**	**0.83**	**183**	**10.90%**	**0.80%**
Teucrium polium	**6.2%**	**28**	**56**	**96**	**110**	**0.34**	**173**	**9.60%**	**0.60%**
***Nigella sativum* (Methanol)**	**13.4%**	**25**	**53**	**80**	**438**	**0.25**	**84**	**8.00%**	**1.10%**
Ceratonia siliqua	**23.2%**	**23**	**43**	**69**	**598**	**0.2**	**0**	**6.90%**	**1.60%**
***Nigella sativum* (Ethanol)**	**20%**	**16**	**29**	**57**	**778**	**0.13**	**87**	**5.70%**	**1.10%**

IV. 3. Antioxidant effects of plant extracts on human erythrocytes exposed to H_2O_2:

IV. 3.1. Effect of plant extracts on lipid peroxidation (i.e. Malonyldialdehyde (MDA) production:

The effect of plant extracts at various concentrations (0.2, 0.4, 0.6 and 0.8 mg/ml) on erythrocyte MDA production, as a marker of lipid peroxidation of erythrocytes exposed to 10 mM H_2O_2 is shown in Fig. 10. The tested plants (at a concentration of 0.8 mg/ml) can be arranged in descending order of strength in decreasing MDA production as follows: *Zingiber officinale* (as the most potent) decreased MDA from a mean of 302.8 nmol/g Hb (with H_2O_2 alone) to a mean of 72.6 nmol/g Hb, followed by *Origanum syriacum* (to a mean of 87.7 nmol/g Hb), *Rosmarinus officinalis* (to a mean of 111.1 nmol/g Hb), *Arbutus andrachne* (to a mean of 111.4 nmol/g Hb), *Pistacia palaestina* (to a mean of 163.6 nmol/g Hb), *Hypericum triquetrifolium* (to a mean of 196.8 nmol/g Hb), *Salvia triloba* (to a mean of 211.8 nmol/g Hb), *Mentha Spp.* (to a mean of 230.1 nmol/g Hb), *Verbena triphylla* (to a mean of 239.1 nmol/g Hb), *Teucrium polium* (to a mean of 252.6 nmol/g Hb), *Nigella sativum* (methanolic) (to a mean of 259.3 nmol/g Hb), *Ceratonia siliqua* (to a mean of 273.9 nmol/g Hb) and *Nigella sativum* (ethanolic extract) (to a mean of 288.0 nmol/g Hb) (see Appendix II).

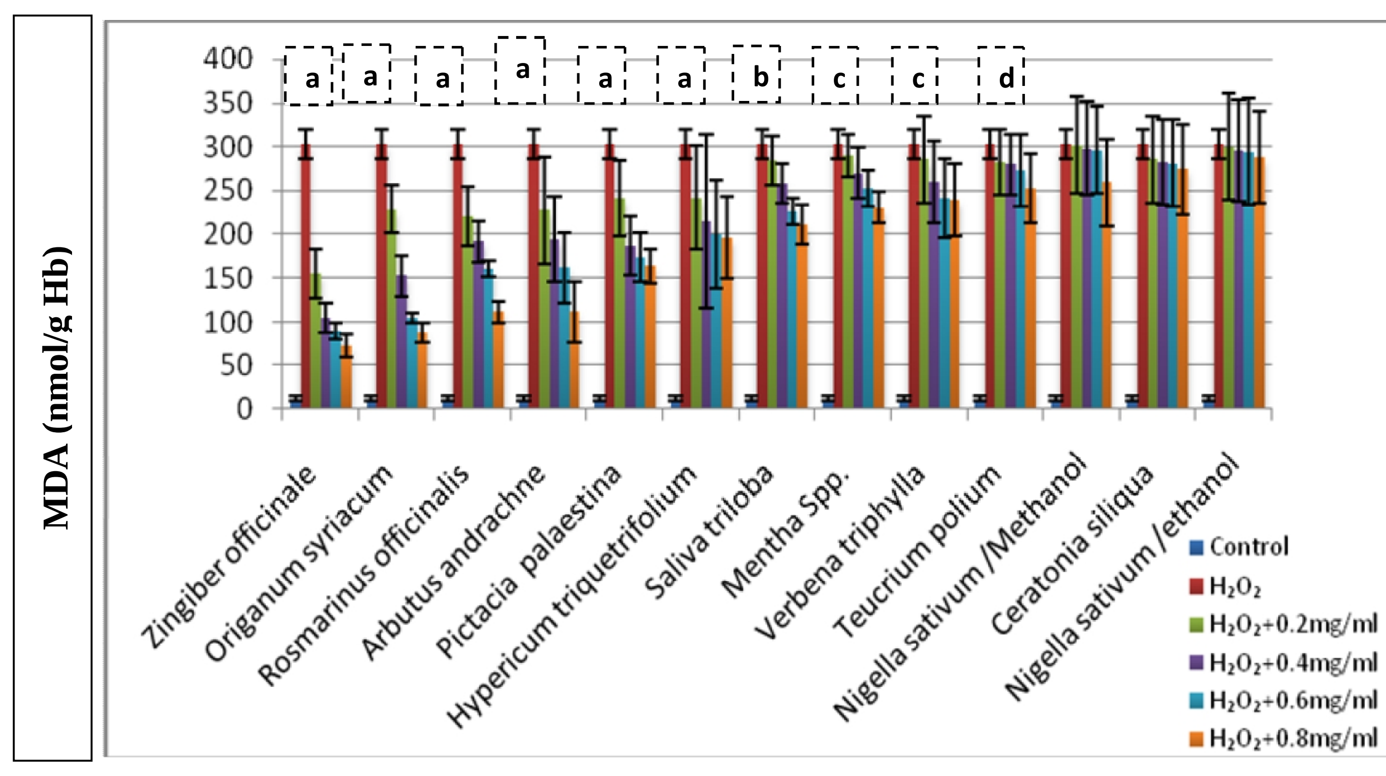


Figure 10: MDA concentration of normal erythrocytes when incubated at 37°C for 60 min in the presence of 10 mM H_2O_2, or in the presence of 10 mM H_2O_2 plus 0.2, 0.4, 0.6 and 0.8 mg/ml from each of *Zingiber officinale, Origanum syriacum, Rosmarinus officinalis, Arbutus andrachne, Pistacia palaestina, Hypericum triquetrifolium, Salvia triloba, Verbena triphylla, Mentha Spp., Teucrium polium, Nigella sativum, Ceratonia siliqua* methanolic extracts and *Nigella sativum* ethanolic extract. Each column represents the mean value and bars represent the Standrd Deviation, a=P< 0.05, at 0.2, 0.4, 0.6 and 0.8mg/ml, compared to treatment with H_2O_2 alone, b=P< 0.05, at 0.4, 0.6 and 0.8mg/ml, compared to treatment with H_2O_2 alone, c =P< 0.05,at 0.6 and 0.8mg/ml, compared to treatment with H_2O_2 alone and d=P< 0.05, at 0.8mg/ml, compared to treatment with H_2O_2 alone.

IV.3.2. Effect of plant extracts on protein oxidation (i.e. Protein Carbonyl production):

The effect of plant extracts at various concentrations (0.2, 0.4, 0.6 and 0.8 mg/ml) on erythrocyte protein carbonyl production, as a marker of protein oxidation of erythrocytes exposed to 10 mM H_2O_2 is shown in Fig. 11. The tested plants (at a concentration of 0.8 mg/ml) can be arranged in descending order of strength in decreasing protein carbonyl production as follows: *Hypericum triquetrifolium* (as the most potent) decreased protein carbonyl from a mean of 1420.5 nmol/g Hb (with H_2O_2 alone) to a mean of 458.2 nmol/g Hb, followed by *Zingiber officinale* (to a mean of 635.2 nmol/g Hb), *Nigella sativum* (to a mean of 727.0 nmol/g Hb), *Rosmarinus officinalis* (to a mean of 775.5 nmol/g Hb), *Teucrium polium* (to a mean of 792.2 nmol/g Hb), *Salvia triloba* (to a mean of 996.3 nmol/g Hb) and *Verbena triphylla* (to a mean of 1165.5 nmol/g Hb). However, *Pistacia palaestina*, *Arbutus andrachne*, *Mentha Spp.*, *Origanum syriacum*, *Ceratonia siliqua* and *Nigella sativum* (ethanol) extracts had no significant effect on protein carbonyl production in erythrocytes exposed to 10 mM H_2O_2 (see Appendix III).

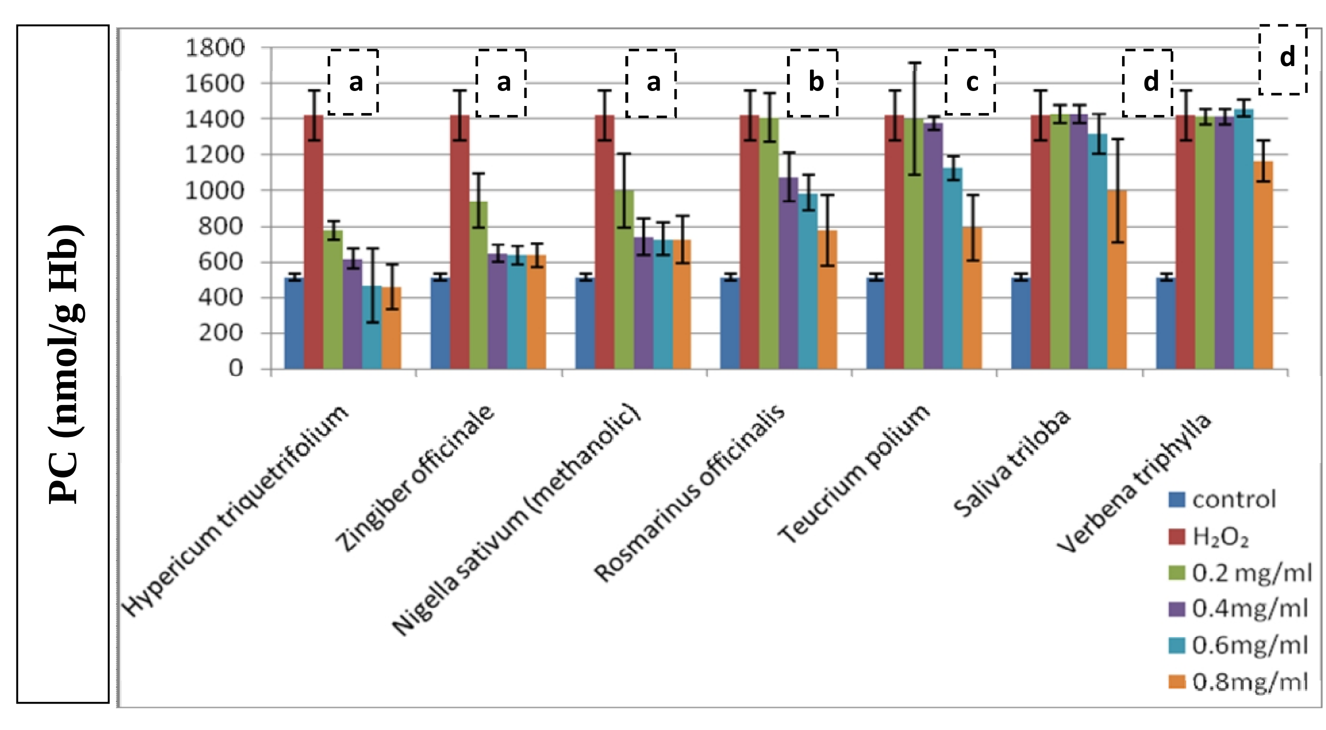


Figure 11: Protein carbonyl concentration of normal erythrocytes when incubated at 37°C for 60 min in the absence or in the presence of 10 mM H_2O_2, or in the presence of 10 mM H_2O_2 plus different concentrations of *Hypericum triquetrifolium, Zingiber officinale, Nigella sativum, Rosmarinus officinalis, Teucrium polium, Salvia triloba* and *Verbena triphylla* methanolic extracts. Each column represents the mean value and bars represent the standrd deviation, a=P< 0.05, at 0.2, 0.4, 0.6 and 0.8mg/ml, compared to treatment with H_2O_2 alone, b=P< 0.05, at 0.4, 0.6 and 0.8mg/ml, compared to treatment with H_2O_2 alone, c =P< 0.05,at 0.6 and 0.8mg/ml, compared to treatment with H_2O_2 alone and d=P< 0.05, at 0.8mg/ml, compared to treatment with H_2O_2 alone.

IV.3.3. Effect of plant extracts on Erythrocyte Reduced Glutathione (i.e. glutathione- depleted erythrocyte):

Incubation of erythrocytes with 10 mM H_2O_2 for 1hr, decreased erythrocyte GSH from a mean of 2.2 mg/g Hb (without H_2O_2) to a mean of 0.87 mg/g Hb (with H_2O_2). Pre-incubation of erythrocytes with the tested plant extracts at 0.2, 0.4, 0.6 and 0.8 mg/ml had no significant effect on GSH compared to treatment with H_2O_2 alone (see Apendix IV, Figs. 1-13). The tested extracts also had no effect on reduced glutathione of erythrocytes incubated without H_2O_2 (see Apendix IV, Figs. 1-13).

IV.3.4. Effect of plant extracts on oxidant hemolysis of erythrocytes:

Oxidative stress also causes hemolysis of erythrocytes exposed to H_2O_2. The effect of plant extracts at various concentrations (0.2, 0.4, 0.6 and 0.8 mg/ml) on hemolysis of erythrocytes exposed to 10 mM H_2O_2 is shown in (Fig. 12). The tested plants (at a concentration of 0.8 mg/ml) can be arranged in descending order of strength in decreasing hymolysis as follows: *Zingiber officinale* (as the most potent) decreased percentage of hymolysis from a mean of 13.7 % (with H_2O_2 alone) to a mean of 1.3 %, followed by *Origanum syriacum* (to a mean of 2 %), *Rosmarinus officinalis* (to a mean of 3.4 %), *Hypericum triquetrifolium* (to a mean of 4.1 %), *Arbutus andrachne* (to a mean of 5.1 %), *Pistacia palaestina* (to a mean of 5.2 %), *Salvia triloba* (to a mean of 5.8 %), *Mentha Spp.* (to a mean of 6.5 %), *Verbena triphylla* (to a mean of 7.0 %) and *Ceratonia siliqua* (to a mean of 10.9 %). Whereas, *Nigella* sativum ethanolic extract and *Teucrium polium* methanolic extract had no significant effect on oxidant hemolysis (see Appendix II).

Unexpectedly, *Nigella sativum* methanolic extract increased the percentage of hemolysis from a mean of 13.7 % (with H_2O_2 alone) to a mean of 14.7% at 0.8 mg/ml (see Appendix II).

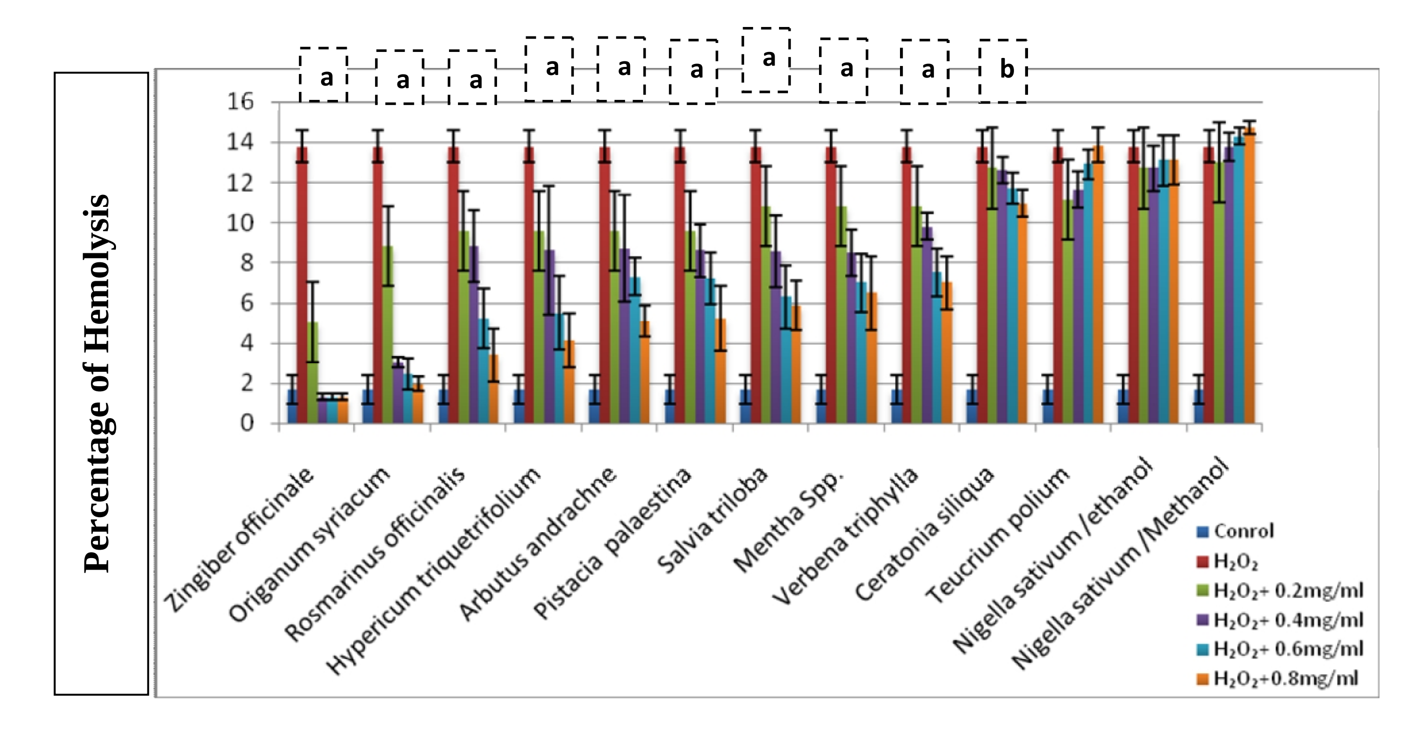


Figure 12: Percentage of Hemolysis of normal erythrocytes when incubated at 37°C for 60 min in the presence of 10 mM H_2O_2, or in the presence of 10 mM H_2O_2 plus different concentrations of *Zingiber officinale, Origanum syriacum, Rosmarinus officinalis, Hypericum triquetrifolium, Arbutus andrachne, Pistacia palaestina*, *Salvia triloba*, *Verbena triphylla, Mentha Spp.* and *Ceratonia siliqua, Teucrium polium* methanolic extract, *Nigella sativum* ethanolic extract and *Nigella sativum* methanolic extract. Each column represents the mean value and bars represent the S.D. (n = 7). a=P< 0.05, at 0.2, 0.4, 0.6 and 0.8mg/ml, compared to treatment with H_2O_2 alone, b=P< 0.05, at 0.8mg/ml, compared to treatment with H_2O_2 alone.

V. Discussion:

V. 1. Chemical antioxidant activities of studied plants:

The results of the present study demonstrated that out of the studied plants, *Pistacia palaestina, Arbutus andrachne and Hypericum triquetrifolium* extracts possess the highest total antioxidant capacity (Fig.4), the highest DPPH free radical scavenging activity (Fig.5) and the highest ferric reducing activity (Fig.6), probably because these extracts contained the highest amount of phenols (Fig.8) and flavonoids (Fig.9). However, *Nigella sativum* (methanolic and ethanolic) extracts, although possessed the highest metal chelating activity (Fig.7), it showed the lowest total antioxidant capacity (Fig.4), the lowest DPPH free radical scavenging activity (Fig.5) and the lowest ferric reducing activity (Fig.6), probably because this extract contained the lowest amount of phenols (Fig.8) and flavonoids (Fig.9). This result coincides with other studies (Luo *et al*., 2002; Conforti *et al*., 2002 and Liu *et al*., 2007) that showed the phenolic and flavonoid contents of extracts of many plants contribute significantly to their total antioxidant capacity.

The ability of plant extracts to scavenge DPPH was assessed on the basis of their IC_{50} values which is defined as the concentration of test material (plant extracts) that decreases the concentration of DPPH free radical to half of its initial value. As shown in Fig. 5, the ability of *Pistacia palaestina* extract to scavenge DPPH free radical was > 40 times effective than that of *Nigella sativum* methanolic extract (IC_{50} = 14.9 and 3013, respectively), this, obviously, is attributed to the higher phenolic and flavonoid contents of the *Pistacia palaestina* compared to *Nigella sativum* methanolic extract (Fig. 8and9). The present study also showed that the antioxidant activity of rosemary methanolic extract is >

10 times effective in DPPH scavenging (IC_{50}= 41) than that of *Nigella sativum* methanolic extract (IC_{50}= 437.7) (Fig.5), which was related to the higher phenolics and flavonoids content of rosemary methanolic extract compared to *Nigella sativum* methanolic extract (Figs.8 and 9). Similar result was also obtained by Erkan *et a*l. (2008) who showed that Rosemary methanolic extract has a much higher phenolic content than *Nigella sativum* methanolic extract, that was resulted in 10 times higher antioxidant activity in DPPH scavenging compared to *Nigella sativum* methanolic extract.

In regard to the antioxidant activity, based on the ferric reducing activity test, it seems that *Pistacia palaestina, Arbutus andrachne* and *Hypericum triquetrifolium* extracts had a highest ferric reducing activity between the screened plants (Fig. 6). Similar result was also found by Tawaha *et al.* (2007) who identified *Arbutus andrachne, Hypericum triquetrifolium* and *Rosmarinus officinalis* as being the best sources for free radical scavenging compounds among the Jordanian plants.

Transition metal ions such as those of copper and iron are important catalysts for the generation of highly reactive hydroxyl radicals via the Fenton reaction in both *in vivo* and *in vitro* systems. Ligands that bind to metal ions can alter the redox potentials of these ions, rendering them catalytically silent. Therefore, the compounds which can act as effective ligands towards sequestering copper and iron ions are also considered to act as antioxidants by intercepting and/or suppressing the radicals formed via metal catalysis (Aruoma, *et al*., 1987). Similarly free hydroxyl groups in some flavonoids by chelating the metal ion may also add to their scavenging ability to free radicals. In the present study the ability to chelate iron ions varied widely for the tested plants – *Rosmarinus officinalis* chelated the least amount of ferrous ions while *Nigella sativum* chelated the most.

However, the highest metal chelating ability of *Nigella sativum* with its lowest antioxidant activities observed in the present study should explain the previously reported anti-lipid peroxidant and anti-protein-degredant activities of *Nigella sativum* towards erythrocytes exposed to H_2O_2 (Suboh *et al*., 2004) as being largely due to its strong metal chelating ability rather than to its scavenging ability towards free radicals.

Considering the overall antioxidant activities, the studied plant extracts were arranged in the following decreasing order of their antioxidant strength expressed as percentage of extract (Table II): *Pistacia palaestina* > *Arbutus andrachne* > *Hypericum triquetrifolium* > *Zingiber officinale* > *Mentha Spp.* > *Rosmarinus officinalis* > *Salvia triloba* > *Verbena triphylla* > *Origanum syriacum* > *Teucrium polium* > *Nigella sativum* methanolic > *Ceratonia siliqua* > *Nigella sativum* ethanolic. When the extractive yield was taken in the account, the studied plants were arranged in the following decreasing order of their total antioxidant capacity expressed as percentage of dry plant (Table II): *Pistacia palaestina* > *Arbutus andrachne* > *Hypericum triquetrifolium* > *Salvia triloba* > *Rosmarinus officinalis* > *Ceratonia siliqua* > *Zingiber officinale* > *Nigella sativum* methanolic > *Origanum syriacum* > *Mentha Spp.* > *Teucrium polium* > *Verbena triphylla*.

V. 2. Antioxidant effects of plant extracts on human erythrocytes exposed to H_2O_2:

Pre- incubation of erythrocytes with methanolic extracts of *Zingiber officinale*, *Origanum syriacum*, *Rosmarinus officinalis*, *Arbutus andrachne*, *Pistacia palaestina*, *Hypericum triquetrifolium*, *Salvia triloba*, *Mentha Spp.* , *Verbena triphylla* and *Teucrium polium* decreased significantly the production of MDA in erythrocytes exposed to 10 mM H_2O_2, indicating anti-lipid-peroxidant activity (Fig. 10). Pre- incubation of erythrocytes with *Nigella sativum* methanolic extract, *Ceratonia siliqua* and *Nigella sativum* ethanolic extract had no significant effect on MDA production, this could be due to their lowest activity for DPPH radical scavenging compared to the other extracts (Fig. 7). Therefore, the present study indicated that the extracts with strong scavenging activity would have a strong anti-lipid-peroxidant activity.

Pre-incubation of erythrocytes with methanolic extracts of *Hypericum triquetrifolium, Zingiber officinale, Nigella sativum, Rosmarinus officinalis, Teucrium polium, Salvia triloba* and *Verbena triphylla* decreased significantly the production of protein carbonyl in erythrocytes exposed to 10 mM H_2O_2, indicating anti-protein-oxidant activity (Fig. 11), that can be explained by the high activity of DPPH radical scavenging (Fig. 7) or by the strong metal chelating ability as in case of *Nigella sativum* methanolic extract (Fig. 9). Unexpectedly *Arbutus andrachne, Pistacia palaestina, Mentha Spp. and Origanum syriacum* although they were strong in free radical scavenging (Fig. 7) and iron chelating abilities (Fig. 9) by chemical assays, but had no anti-protein-oxidant activity in

erythrocytes. This may indicate the complexity of the anti-protein-oxidant activity that could be due to properties other than scavenging activity or metal chelating ability, which can not be determined by the present study, but could be speculated as being due to the ability of the antioxidant to dissolve readily in the hydrophobic medium of the haem pocket of hemoglobin in which resides the metal iron that suppose to be responsible for protein oxidantion by Fenton reaction.

The present study also showed that the tested extracts had no significant effect on erythrocyte reduced glutathione, indicating that the antioxidant activity of the tested extracts is not mediated through increasing the erythrocyte reduced glutathione at least in the present *in vitro* experimental condition which is supposed to be not suitable for the reproduction of erythrocyte reduced glutathione.

Incubation of erythrocytes with H_2O_2 caused hemolysis that could be due to the increased rigidity of erythrocyte membrane, probably due to lipid peroxidation. Pre-incubation of erythrocytes with the tested plant extracts that showed anti-lipid peroxidant activity (Fig. 10) also decreased oxidant hymolysis (Fig. 12), indicating that the anti-lipid peroxidant activity was probably responsible for the prevention of hemolysis. Unexpectedly, *Nigella sativum* methanolic extract at high concentration (0.8 mg/ml) increased the percentage of oxidant hemolysis of erythrocytes exposed to H_2O_2, probably due to the high content of saponins (hemolyzing agents) that were reported to make the main chemical constituents of the polar fraction of methanolic extract of *Nigella sativum* (Kumara and Huat, 2001; Sparg *et al.*, 2004: Elbandy *et al.*, 2009 and Mehta *et al.*, 2009).

VI. Summary and Conclusions:

- In this study12 plant species were examined: *Teucrium polium* (Aerial parts), *Nigella sativum* (seeds), *Zingiber officinale* (rhizomes), *Rosmarinus officinalis* (leaves), *Verbena triphylla* (leaves), *Mentha Spp.* (leaves), *Salvia triloba* (leaves), *Origanum syriacum* (leaves), *Hypericum triquetrifolium* (whole plant), *Ceratonia siliqua* (fruits), *Pistacia palaestina* (leaves) and *Arbutus andrachne* (leaves).
- Extraction yields (g extract/ 100g dry plant part) for each plant species were obtained and the most efficient extraction yield by methanol was obtained for *Arbutus andrachne* (31.0%), followed by *Pistacia palaestina* (30.5%), *Hypericum triquetrifolium* (27%), *Ceratonia siliqua* (23.2%), *Nigella sativum* (ethanolic) (20%), *Salvia triloba* (15.5), *Nigella sativum* (methanolic) (13.4%), *Rosmarinus officinalis* (12%), *Origanum syriacum* (7.1%), *Teucrium polium* (6.2%), *Verbena triphylla* (4.1%), *Mentha Spp.* (3.9%).
- The studied plant extracts were arranged in decreasing order of their total phenol content (mg Catechol equivalent/g extract) as follows: *Pistacia palaestina* > *Hypericum triquetrifolium* >*Arbutus andrachne* > *Zingiber officinale* > *Mentha Spp*. > *Rosmarinus officinalis* > *Salvia triloba* > *Verbena triphylla* > *Origanum syriacum* > *Teucrium polium* > *Nigella sativum/*methanol > *Ceratonia siliqua* > *Nigella sativum/*ethanol
- The studied plant extracts were arranged in decreasing order of their total flavonoid content (mg Rutin equivalent/g extract) as follows: *Hypericum triquetrifolium* > *Pistacia palaestina* > *Arbutus andrachne* > *Mentha Spp.* > *Zingiber officinale* > *Rosmarinus officinalis* > *Salvia triloba* >*Verbena triphylla* > *Origanum syriacum*

> *Teucrium polium* > *Nigella sativum/*methanol > *Ceratonia siliqua* > *Nigella sativum/*ethanol.

- The studied plant extracts were arranged in decreasing order of their total antioxidant capacity (µg AA equivalent/mg extract) as follows: *Pistacia palaestina* > *Arbutus andrachne* > *Hypericum triquetrifolium* > *Zingiber officinale* > *Mentha Spp*. > *Rosmarinus officinalis* > *Salvia triloba* > *Verbena triphylla* > *Origanum syriacum* > *Teucrium polium* > *Nigella sativum/*methanol > *Ceratonia siliqua* > *Nigella sativum/*ethanol.
- The studied plant extracts were arranged in decreasing order of their scavenging ability towards DPPH free radical as follows: *Pistacia palaestina* > *Arbutus andrachne* >*Hypericum triquetrifolium* > *Mentha Spp*. >*Salvia triloba*> *Rosmarinus officinalis* > *Verbena triphylla* > *Zingiber officinale* > *Origanum syriacum* > *Teucrium polium* > *Nigella sativum/*methanol >*Nigella sativum/*ethanol> *Ceratonia siliqua*.
- The studied plant extracts were arranged in decreasing order of their ferric reducing activity as follows: *Pistacia palaestina* > *Hypericum triquetrifolium* > *Arbutus andrachne* > *Zingiber officinale* > *Mentha Spp.* > *Rosmarinus officinalis* > *Salvia triloba* > *Verbena triphylla* > *Origanum syriacum* > *Teucrium polium* > *Nigella sativum/*methanol > *Ceratonia siliqua* > *Nigella sativum/*ethanol.
- The studied plant extracts were arranged in decreasing order of their metal chelating activity as follows: *Nigella sativum/* methanolic extract > *Nigella sativum/* ethanolic extract > *Pistacia palaestina* > *Arbutus andrachne* > *Hypericum*

triquetrifolium > *Teucrium polium* > *Mentha Spp.* > *Origanum syriacum* > *Zingiber officinale* > *Verbena triphylla* > *Salvia triloba* > *Rosmarinus officinalis*.

- *Nigella sativum* had the lowest antioxidant activities, despite its highest metal chelating activity, indicating that it might exert its antioxidant activity in biological systems largely via its metal chelating property rather than free radical scavenging.
- The studied plants were arranged in decreasing order of their *in vitro* anti-lipid-peroxidant activity in human erythrocyte as follows: *Zingiber officinale* > *Origanum syriacum* > *Rosmarinus officinalis* > *Arbutus andrachne* > *Pistacia palaestina* > *Hypericum triquetrifolium* > *Salvia triloba* > *Mentha Spp.* > *Verbena triphylla* > *Teucrium polium* > *Nigella sativum* methanolic > *Ceratonia siliqua* > *Nigella sativum* ethanolic.
- Some of studied plants are arranged in decreasing order of their *in vitro* anti-protein-oxidant activity in human erythrocyte as follows: *Hypericum triquetrifolium* > *Zingiber officinale* > *Nigella sativum* > *Rosmarinus officinalis* > *Teucrium polium* > *Salvia triloba* > *Verbena triphylla*.
- Unexpectedly, some plants such as *Arbutus andrachne*, *Pistacia palaestina*, *Mentha Spp. and Origanum syriacum* although they were strongest in scavenging activity and strongest in iron chelating activity next to *Nigella sativum* but had no anti-protein-oxidant activity in erythrocyte which indicates the complexity of the anti-protein-oxidant activity that could be due to properties other than scavenging activity or metal chelating ability, which can not be determined in the present study, but could be speculated as being due to the ability to dissolve in the hydrophobic medium of the haem pocket in which resides the metal iron.

VII. Recommendation:

- Some potential species such as *Pistacia palaestina*, *Arbutus andrachne* and *Hypericum triquetrifolium* exhibited the highest phenol content and antioxidant activities in this study. These species seem to be important new natural sources for antioxidants that need to be investigated further.
- *Nigella sativum* could be used as a good source for new agents for iron chelating drugs which needs further investigation in this regard.

VIII. References:

Aburjai, T. Hudaib, M. Tayyema, R. Yousef, M. Qishawi, M. (2007), Ethnopharmacological survey of medicinal herbs in Jordan, the Ajloun Heights region. **Journal of Ethnopharmacology**, 110, 294 304.

Afifi, F.U. and Abu-Irmaileh, B. (2000), Herbal medicine in Jordan with special emphasis on less commonly used medicinal herbs. **Journal of Ethnopharmacology**, 72, 101–110.

Alali, F. Tawaha, Kh. El-Elimate, T. Syouf, M. EL-Fayad, M. Abulaila, Kh. Nielsen, S.J. Wheaton, W. Falkinham, J. and Oberlies, N. (2007), Antioxidant activity and total phenolic content of aqueous and methanolic extracts of Jordanian plants: an ICBG project. **Natural Product Research**, 21, 1121-1131.

Al-Eisawi, D.M. (1982), List of Jordan Vascular plants. **Mitteilungen Botanik Munchen**, 18, 79–182.

Ames, B.N. Shigenaga, M.K. and Hagen, T.M. (1993), Oxidants, antioxidants, and the degenerative diseases of aging. **Proceedings of the National Academy of Sciences of the USA**, 90, 7915- 7922.

Amici, A. Levin, R. L. Tsai, L. Tsai, L. and Stadtman, E. R. (1989), Conversion of amino acid residues in proteins and amino acid homopolymers to carbonyl derivatives by metal-catalyzed oxidation reaction. **Journal of Biological Chemistry**, 264, 3341-3346.

Antolovich, M. Prenzler, P.D. Patsalides, E. and Robards, K. (2002), Methods for testing antioxidant activity. **The Analyst**, 127, 183-198.

Aruoma, O. I. (1998), Free radical, oxidative stress, and antioxidants in human health and disease. **Journal of the American Oil Chemists Society**, 75, 199–212.

Aruoma, O. I., Grootveld, M., and Halliwell, B. (1987), The role of iron in ascorbate dependent deoxyribose degradation. Evidence consistent with a site-specific hydroxyl radical generation caused by iron ions bound to the deoxyribose molecule. **Journal of Inorganic Biochemistry**, 29, 289–299.

Avallone, R. Plessi, M. Baraldi, M. Monzani, A. (1997), Determination of Chemical Composition of Carob (Ceratonia siliqua): Protein , Fat , Carbohydrates , and Tannins. **Journal of Food Composition and Analysis,** 10, 166-172.

Berlett, B.S. and Stadtman, E.R. (1997), Protein oxidation in aging, disease, and oxidative stress. **Journal of Biological Chemistry**, 272, 20313-20316.

Berman, R.S. and Martin, W. (1993), Arterial endothelial barrier dysfunction: actions of homocysteine and the hypoxanthine-xanthine oxidase free radical generating system. **British Journal of Pharmacology**, 108, 920-926.

Block, G. and Patterson, B. (1992), Fruits, vegetables and cancer prevention: a review of the epidemiological evidence. **Nutrition and Cancer**, 18, 1-29.

Bloiss, M.S. (1958), Antioxidant determinations by the use of stable free radical. **Nature**, 181, 1199-1200.

Bonnefont-Rousselot, D. Bastard, J.P. Jaudon, M.C. Dellattre, J. (2000), Consequences of the diabetic status on the oxidant/antioxidant balance. **Journal of Diabetes and Metabolism**, 26, 163-176.

Boskou, G. Salta, F. Chrysostomou, S. Mylona, A. Chiou, A. and Andrikopoulos, N.K. (2006), Antioxidant capacity and phenoic profile of table olives from Greek market. **Food Chemistry**, 94, 558-564.

Brash, A.R. (1999), Lipoxygenases: occurrence, functions, catalysis, and acquisition of substrate. **Journal of Biological Chemistry**, 274, 23679-23682.

Cai, Y. Luo, Q. Sun, M. and Corke, H. (2004), Antioxidant activity and phenolic compounds of 112 traditional Chinese medicinal plants associated with anticancer. **Life Science**, 74, 2157-2184.

Cao, G. Sofic, E. R. and Prior, R.L. (1996), Antioxidant capacity of tea and common vegetables. **Journal of Agricultural and Food Chemistry**, 44, 3426-3431.

Chance, B. Sies, H. and Boveris, A. (1979), Hydroperoxide metabolism in mammalian organs. **Physiology Review**, 59, 527-605.

Cheikh-Rouhou Salma, Besbes Souhail, Hentati Basma, Blecker Christophe, Deroanne Claude and Attia Hamadi (2007), *Nigella sativa* L.: Chemical composition and physicochemical characteristics of lipid fraction, **Food Chemistry**, 101, 673-681.

Conforti, F. A.Statti, G. Tundis, R. Menichini, F. Houghton, P. (2002), Antioxidant activity of methanolic extract of *Hypericum triquetrifolium* Turra aerial part. **Fitoterapia**, 73, 479–483.

Davies, K. J. A. and Goldberg, A. L. (1987), Oxygen radicals stimulate intracellular proteolysis and lipid peroxidation by independent mechanism in erythrocytes. **Journal of Biological Chemistry**, 262, 8220-8226.

Devasagayam, T.P. Steenken, S. Obendorf, M.S. Schulz, W.A. and Sies, H. (1991), Formation of 8-hydroxy(deoxy)guanosine and generation of strand breaks at guanine residues in DNA by singlet oxygen. **Biochemistry**, 30, 6283-6289.

Dinis, T. C. P. Madeira, V. M. C. and Almeida, L. M. (1994), Action of phenolic derivates (acetaminophen, salicylate, and 5- aminosalicylate) as inhibitors of membrane lipid peroxidation and as peroxyl radical scavengers. **Archives of Biochemistry and Biophysics.**, 315, 161- 169.

Djeridane, A. Yousfi, Nadjemi, M. B. Boutassouna, D. Stocker, P. Vidal, N. (2006), Antioxidant activity of some Algerian medicinal plant extracts containing phenolic compounds. **Food Chemistry**, 97, 654-660.

Duh, P. D. and Yen, G. C. (1997), Antioxidant activity of three water extracts. **Food Chemistry**, 60, 639-645.

Elbandy, M. Kang, O. Kwon, D. and Rho, J. (2009), Two new antiinflammatory triterpene saponins from the egyptian medicinal food black cumin (Seeds of *Nigella sativum*). **Bulletin of Korean Chemistry Society,** 30, 1811-1816.

Ellman, G. L.(1951), Tissue Sulfhydryl (-SH) Groups. **Archive of Biochemistry and Biophysiology**, 82, 70-77.

El-Sayed, N. H. Khalifa, T. I. Ibrahim, M. T. and Mabry, T. J. **(**2001), Constituents from *Salvia triloba* ,**Fitoterapia**, 72 850-853.

Ekstrom, G. and Ingelman-Sundberg, M. (1989), Rat liver microsomal NADPH supported oxidase activity and lipid peroxidation dependent on ethanol-inducible cytochrome P-450 (P-450IIE1). **Biochemistry and Pharmacology**, 38, 1313-1319.

Erkan, N. Ayranci, G. Ayranci, E. (2008), Antioxidant activities of rosemary (*Rosmarinus Officinalis* L.) extract, black seed (*Nigella sativum* L.) essential oil, carnosic acid, rosmarinic acid and sesamol. **Food Chemistry**, 110, 76–82.

Flamini Guido, Bader Ammar, Luigi Cioni Pier, Katbeh-Bader Ahmad, and Morelli Ivano (2004), Composition of the Essential Oil of Leaves, Galls, and Ripe and Unripe Fruits of Jordanian *Pistacia palaestina* Boiss, **Journal of Agricultural Food Chemistry**, 52, 572–576.

Fiorentino, A. Castaldi, S. D‘Abrosca, B. Natale, A. Carfora, A. Messere, A. P., Monaco. (2007) Polyphenols from the hydroalcoholic extract of Arbutus unedo living in a monospecific Mediterranean woodland. **Biochemical Systematics and Ecology**, 35, 809-811.

Freeman, B. A. and Crapo, J. D. (1982), Biology of disease: free radicals and tissue Injury. **Laboratory Investigation**, 47, 412-426.

Fridovich, I. (1970), Quantitative aspects of the production of superoxide anion radical by milk xanthine oxidase. **Journal of Biological Chemistry**, 245, 4053-4057.

Gutteridge, J.M.C. and Stocks, J. (1981), Caeruloplasmin: physiological and pathological perspectives. **Critical Reviews in Clinical Laboratory Science**, 14, 257-329.

Halliwell, B. (1989), Protection against tissue damage in vivo by desferrioxamine: What is its mechanism of action?. **Free Radical Biology and Medicine**, 7, 645–651.

Halliwell, B. (1991), Reactive oxygen species in living systems: source, biochemistry, and role in human disease. **American Journal of the Medical Sciences**, 91, 14S-22S.

Halliwell, B. (1993), The role of oxygen radicals in human disease, with particular reference to the vascular system. **Haemostasis**, 23, 118-126.

Halliwell, B. (1994), Free radicals, antioxidants, and human disease: curiosity, cause, or consequence? **Lancet**, 344, 721-724.

Halliwell, B. (1995), How to characterize an antioxidant: an update. **Biochemical Society Symposia**, 61, 73–101.

Halliwell, B. (1999), Antioxidant defence mechanisms: from the beginning to the end (of the beginning). **Free Radical Research**, 31, 261-272.

Halliwell, B. and Chirico, S. (1993), Lipid peroxidation: its mechanism, measurement, and significance. **American Journal of Clinical Nutrition**, 57, 715S-724S.

Halliwell, B. and Gutteridge, J.M.C.(1984), Lipid peroxidation, oxygen radical, cell damage and antioxidants therapy. **Lancet**, i, 1396-1398.

Halliwell, B. Gutteridge, J.M. and Cross, C.E. (1992), Free radicals, antioxidants, and human disease: where are we now?. **Journal of Laboratory and Clinical Medicine**, 119, 598-620.

Halliwell, B. and Gutteridge, JMC. (1999), Free Radicals In Biology And Medicine. In **Free Radicals In Biology and Medicine**, Oxford University Press, UK, pp. 10-50.

Harman, D. (1981), The aging process. **Proceedings of the National Academy of Sciences of the USA**, 78, 7124-7128.

Harman, D. (2001), Aging: overview. **Annals of the New York Academy of Sciences**, 928: 1-21.

Heim, K.E. Tagliaferro, A.R. Bobilya, D.J. (2002), Flavonoid antioxidants: chemistry, metabolism and structue-activity relationships. **The Journal of Nutritional Biochemistry**, 13, 572-584.

Irhimeh, M. R. (2001), Effects of oxygen radicals on membrane permeability and deformability of erythrocytes. **M. Sc. Thesis, University of Jordan, Amman, Jordan**.

Kahl, R. and Kappus, H. (1993), Toxicology of the synthetic antioxidants BHA and BHT in comparison with the natural antioxidant vitamin E. **Zeitschrift fur Lebensmittel Untersuchung und -Forschung**, 196(4), 329–338.

Katalinic, V. Milos, Kulisic, M. T. Jukic, M. (2006), Screening of 70 medicinal plant extracts for antioxidant capacity and total phenols. **Food Chemistry**, 94, 550-557.

Kasai, H. (1997), Analysis of a form of oxidative DNA damage, 8-hydroxy-2' deoxyguanosine, as a marker of cellular oxidative stress during carcinogenesis. **Mutation Research**, 387, 147-163

Kumara, S.S.M. Huat, B.T.K. (2001), Extraction, isolation and characterization of antitumor principle, θ-hederin, from the seeds of *Nigella sativum*. **Planta Medica**, 67, 29-32.

Kuthan, H. and Ullrich, V. (1982), Oxidase and oxygenase function of the microsomal cytochrome P450 monooxygenase system. **European Journal of Biochemistry**, 126, 583-588.

Jolad Shivanand D. Clark Lantz R. Jie Chen Guan Bates Robert B. and Timmermann Barbara N. (2005), Commercially processed dry ginger (*Zingiber officinale*): Composition and effects on LPS-stimulated PGE_2 production, **Phytochemistry** 66, 1614-1635.

Lako, J. Trenerry, V.C. M. Wahlqvist, N. Wattanapenpaiboon, S. Sotheeswaran, R. (2007), Phytochemical flavonols, carotenoids, and the antioxidant properties of a wide selection of fijian fruit, vegetables and other readily available foods. **Food Chemistry**, 101, 1727-1741.

Lee, J.K. N. and Min, D.B. (2004), Reactive Oxygen Species, Aging, and Antioxidative Nutraceuticals. **Comprehensive Reviews in Food Science and Food Safety**, 3, 21- 33.

Liu, L. and Meydani, M. (2002), Combined vitamin C and E supplementation retards early progression of arteriosclerosis in heart transplant patients. **Nutrition Reviews**, 60, 368-371.

Liu, X. Dong, M. Chen, X. Jiang, M. Lv, X. and Yan, G. (2007), Antioxidant activity and phenolics of an endophytic Xylaria sp. from Gingko biloba. **Food Chemistry**, 105, 548–554.

Lukas Brigitte, Schmiderer Corinna, Franz Chlodwig and Novak Johannes (2009), Composition of Essential Oil Compounds from Different Syrian Populations of *Origanum syriacum* L. (Lamiaceae), **Journal of Agricultural Food Chemistry**, 57,1362–1365.

Luo, X.D. Basile, M.J. and Kennelly, E.J. (2002), Polyphenolic antioxidants from the fruits of *Chrysophyllum cainito* L. (star apple). **Journal of Agricultural Food Chemistry**, 50, 1379-1382.

Machlin, L.J. and Bendich, A. (1987, Free radical tissue damage: protective role of antioxidant nutrients. **FASEB Journal**, 1, 441-445.

Maiorino, M. Chu, F.F. Ursini, F. Davies, K.J.A., Doroshow, J.H. and Esworthy, R.S. (1991), Phospholipid hydroperoxide glutathione peroxidase is the 18-kDa selenoprotein expressed in human tumor cell lines. **Journal of Biological Chemistry**, 266, 7728-7732.

Manach, C. Scalbert, A. Morand, C. Remesy, C. and Jimenez, L. (2004), Polyphenols: food sources and bioavailability. **American Journal of Clinical Nutrition**, 79, 727-747.

Marina D. Soković , Jelena Vukojević , Petar D. Marin , Dejan D. Brkić , Vlatka Vajs and Leo J.L.D. van Griensven (2009), Chemical Composition of Essential Oils of *Thymus* and *Mentha* Species and Their Antifungal Activities **Molecules**, 14, 238-249.

Mehta, B. K. Mehta, P. and Gupta, M. (2009), A new naturally acetylated triterpene saponin from Nigella sativum. **Carbohydrate Research**, 344, 149–151.

Menichini Federica, Conforti Filomena, Rigano Daniela, Formisano Carmen, Franco Piozzi, Senatore Felice (2009), Phytochemical composition, anti-inflammatory and antitumour activities of our Teucrium essential oils from Greece, **FoodChemistry**, 115, 679–686.

Meydani, M. (2000), Vitamin E and prevention of heart disease in high-risk patients. **Nutrition Reviews**, 58, 278-281.

Mezzetti, A. Di Ilio, C. Calafiore, A.M. Aceto,A. Marzio, L. Frederici,G. and Cuccurullo, F. (1990), Glutathione peroxidase, glutathione reductase and glutathione transferase activities in the human artery, vein and heart. **Journal of Molecular and Cellular Cardiology**, 22, 935-938.

Miliauskas, G. Venskutonis, P. R. Van-beek, T. A. (2004), Screening of radical scavenging activity of some medicinal and aromatic plant extracts. **Food Chemistry**, 85, 231-237.

Miller, A .L. (1996), Antioxidant flavonoids: structure, function and clinical usage. **Alternative Medicine Review**, 1, 103.

Mohazzab, K. M. Kaminski, P. M. and Wolin, M.S. (1994), NADH oxidoreductase is a major source of superoxide anion in bovine coronary endothelium. **American Journal of Physiology**, 266, H2568-H2572.

Mulinacci, N. Bardazzi, C. Romani, A. Pinelli, P.Vincieri, F. F. and Costantini, A. (1999), HPLC-DAD and TLC-densitometry for quantification of hypericin in *Hypericum perforatum* L. extracts. **Chromatographia**, 49, 197-201.

Ness, A. R. and Powles, J. W. (1997), Fruit and vegetables and cardiovascular disease: a review. **International Journal of Epidemiology**, 26, 1-13.

Niki, E. Kawakami A. Saito, M. Yamamoto, Y. Tsuchiya, J. and Kamiya, Y. (1985), Effect of phytyl side chain of vitamin E on its antioxidant activity. **Journal of Biological Chemistry**, 260, 2191-2196.

Nishiki, K. Jamieson, D. Oshino, N. and Chance, B. (1976), Oxygen toxicity in the perfused rat liver and lung under hyperbaric conditions. **Biochemistry Journal**, 160, 343-355.

Noguchi, N. and Niki, E. (1998), Dynamics of vitamin E action against LDL oxidation. **Free Radical Research**, 28, 561-572.

Oliver, C.N. Ahn, B-W. Moerman, E.J. Goldstein, S. and Stadtman, E.R. (1987), Agerelated changes in oxidized proteins. **Journal of Biological Chemistry**, 262, 5488-5491.

Oran, S. (1994), Genetic resources of medicinal plants in Jordan. In: Jaradat, A. (Ed.), Proceedings of the National Seminar on Plant Genetic Resources of Jordan. August 2–4. Regional Office, West Asia and North Africa.

Oran, S.A. Al-Eisawi, D.M. (1998), Check-list of medicinal plants in Jordan. Dirasat. **Medical and Biological Sciences**, 25, 84–112.

Oyaizu, M. (1986), Studies on product of browning reaction prepared from glucosamine. **Japanese Journal of Nutrition**, 44, 307- 315.

Palinski, W. Rosenfeld, M.E. Yla-Herttuala, S. Gurtner, G.C. Socher, S.S. Butler, S.W. Parthasarathy, S. Carew, T.E. Steinberg, D. and Witztum, J.L. (1989), Low density lipoprotein undergoes oxidative modifications *in vivo***. Proceedings of the National Academy of Sciences of the United States of America**, 86, 1372-1376.

Prieto, P. Pineda, M. and Aguilar, M. (1999), Spectrophotometric quantification of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application of vitamin E. **Analytical Biochemistry**, 269, 337-341.

Prior, L. P. Wu, X. and Schaich K. (2005), Standardized methods for the determination of antioxidant capacity and phenolic in foods and dietary supplements. **Agricultural Food Chemistry**, 76, 245-256.

Puntarulo, S. and Cederbaum, A. I. (1998), Production of reactive oxygen species by

microsomes enriched in specific human cytochrome P450 enzymes. **Free Radical Biology and Medicine**, 24, 1324-1330.

Reznick, A. and Packer, L. (1994),Oxidative damage to proteins:Spectrophotometric method for carbonyl assay. **Methods in Enzymology**, 233, 357-363.

Rice-Evans, C.A. Miller, N.J. and Paganga, G. (1996), Structure-antioxidant activity relationships of flavonoids and phenolic acids. **Free Radical Biology and Medicine**, 20, 933-956.

Rice-Evans, C.A. Miller, N. J. Paganga, G. (1997), Antioxidant properties of phenolic compouns. **Trends in Plant Science Reviews**, 2, 1360-1385.

Ross, R. (1999), Atherosclerosis-an inflammatory disease**. New England Journal of Medicine**, 340, 115-126.

Serron, S.C. Dwivedi, N. and Backes, W. L. (2000), Ethylbenzene induces microsomal oxygen free radical generation: antibody-directed characterization of the responsible cytochrome P450 enzymes. **Toxicology and Applied Pharmacology**, 164, 305-311.

Sies, H. (1997), Oxidative stress: oxidants and antioxidants. **Experimental Physiology**, 82, 291- 295.

Sies, H. and Cadenas, E. (1985), Oxidative stress: damage to intact cells and organs. **Philosophical Transactions of the Royal Society of London, Series B: Biological Sciences**, 311, 617-631.

Sies, H. and Summer, K.H. (1975), Hydroperoxide-metabolizing systems in rat liver. **European Journal of Biochemistry**, 57, 503-512.

Sies, H. Stahl, W. and Sevanian, A. (2005), Nutritional, dietary and postprandial oxidative stress. **Journal of Nutrition**, 135, 969–972.

Shahidi, F. and Wanasundara, P.K. (1992), Phenolic antioxidants. **Critical Reviews in Food Science and Nutrition**, 32, 67-103.

Sparg, S. G. Light, M. E. van Staden, J. (2004), Review of Biological activities and distribution of plant saponins. **Journal of Ethnopharmacology**, 94, 219–243.

Srour, M. A. Bilto, Y. Y. Juma, M. Irhimeh, M. R. (2000), Exposure of human erythrocytes to oxygen radicals causes loss of deformability, increased osmotic fragility, lipid peroxidation and protein degradation. **Clinical Hemorheology and Microcirculation**, 23, 13–21.

Stadtman, E.R. (2001), Protein oxidation in aging and age-related diseases. **Annals of the New York Academy of Sciences**, 928, 22-38.

Stadtman, E.R. and Levine, R.L.(2003), Free radical-mediated oxidation of free amino acids and amino acid residues in proteins. **Amino Acids**, 25, 207-218.

Starke-Reed, P.E. and Oliver, C.N.(1989), Protein oxidation and proteolysis during aging and oxidative stress. **Archives of Biochemistry and Biophysics**, 275, 559-567.

Steinberg, D.(1997), Low density lipoprotein oxidation and its pathobiological significance. **Journal of Biological Chemistry**, 272, 20963-20966.

Stief, T.W. (2000), The blood fibrinolysis/deep-sea analogy: a hypothesis on the cell signals singlet oxygen/photons as natural antithrombotics. **Thrombosis Research**, 99, 1-20.

Srour, M, A. (1998), Evaluation of different methods used to measure the oxidant stress on human erythrocytes. M. Sc. Thesis, University of Jordan, Amman, Jordan.

Srour, M, A., Bilto, Y. Y., Juma, M. and Irhimeh, M. R. (2000), Exposure of human erythrocytes to oxygen radicals causes loss of deformability, increased osmotic fragility, lipid peroxidation and protein degradation. **Clinical Hemorheology and Microcirculation**, 23, 1-9.

Stocks, J, and Dormandy, T. L. (1971), The autoxidation of human red cell lipids induced by hydrogen peroxide. **British Journal of Hematology**, 20, 95–111.

Stevenson, D. E. and Hurst R. D.(2007), Polyphenolic phytochemicals – just antioxidants or much more? **Journal of Cellular and Molecular Life Sciences**, 64, 2900 – 2916.

Suboh, S. M. (2002), The antioxidant actions of selected medicinal plants on erythrocytes. M. Sc. Thesis, University of Jordan, Amman, Jordan.

Suboh, S. M. Bilto, Y. Y. Aburjai, T. A. (2004), Protective effects of selected medicinal plants against protein degradation, lipid peroxidation and deformability loss of oxidatively stressed human erythrocyte. **Phytotherapy Research**, 18, 280- 284.

Tawaha, Kh. Alali, F. Gharaibeh, M. Mohammad, M. and El-Elimate, T. (2007), Antioxidant activity and total phenolic content of selected Jordanian plant species. **Food Chemistry**, 104, 1372-1378.

Tomas-Barberan, E A. Grayer-B arkmeijer, R. J., Gil M. L. Harborne, J.B.,**Phytochemistry** (1988),27, 2631.

Touafek, O. Nacer, A. Kabouche, A. Kabouche, Z. and Bruneau ,C. chemical composition of the essential oil of *Rosmarinus officinalis* cultivated in the algerian sahara **Chemistry of Natural Compounds**,40,28-29.

Turrens, J. F. and Boveris, A. (1980), Generation of superoxide anion by the NADH dehydrogenase of bovine heart mitochondria. **Biochemical Journal**, 191, 421-427.

Villano, D. Fernandez-Pachon, M.S. Troncosa, A.M. Garcia-Parilla, M.C. (2004), The antioxidant activity of wines determined by the ABTS•+ method: Influence of sample dilution and time. **Talanta**, 64, 501-509.

Virag, L. Szabo, E. Gergely, P. and Szabo, C. (2003), Peroxynitrite-induced cytotoxicity: mechanism and opportunities for intervention. **Toxicology Letters**, 140-141, 113-124.

Wu, X. Beecher, G. R. Holden, J. M. Haytowitz, D. B. Gebhardt, S. E. and Prior, R. L. (2004), Lipophilic and hydrophilic antioxidant capacities of common foods in the United States. **Journal of Agricultural and Food Chemistry**, 52, 4026-4037.

Yeldandi, A.V. Rao, M.S. and Reddy, J.K. (2000), Hydrogen peroxide generation in peroxisome proliferator-induced oncogenesis. **Mutation Research**, 448, 159-177.

Yin, D. and Chen, K. (2005), The essential mechanisms of aging: Irreparable damage accumulation of biochemical side-reactions. **Experimental Gerontology Journal**, 40, 455-465.

Yla-Herttuala, S. Palinski, W. Rosenfeld, M.E. Steinberg, D. and Witztum, J.L. (1990), Lipoproteins in normal and atherosclerotic aorta. **Europeon Heart Journal**, 11, 88-99.

Zhu, H., Bannenberg, G.L., Moldeus, P. and Shertzer, H.G. (1994), Oxidation pathways for the intracellular probe 2’,7’-dichlorofluorescein. **Archives of Toxicology**, 68, 582-587.

Appendix I

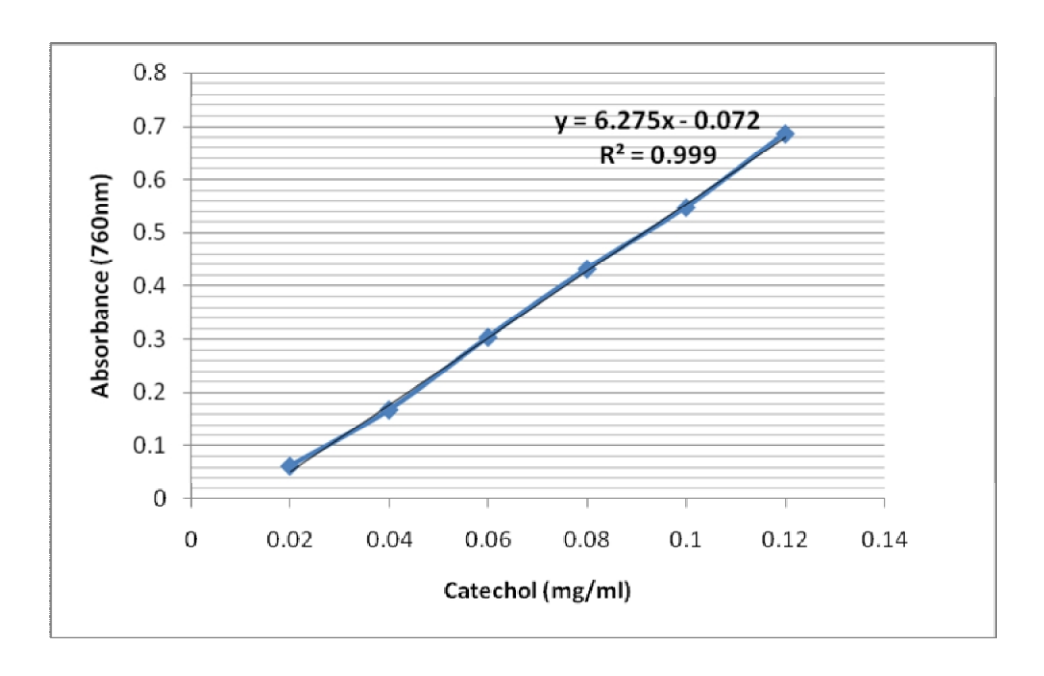


Figure (1): Calibration curve for the determination of total phenolic compound contents in extracts, calculated by linear regression analysis. Concentrations of catechol (x) versus the absorbance (y).

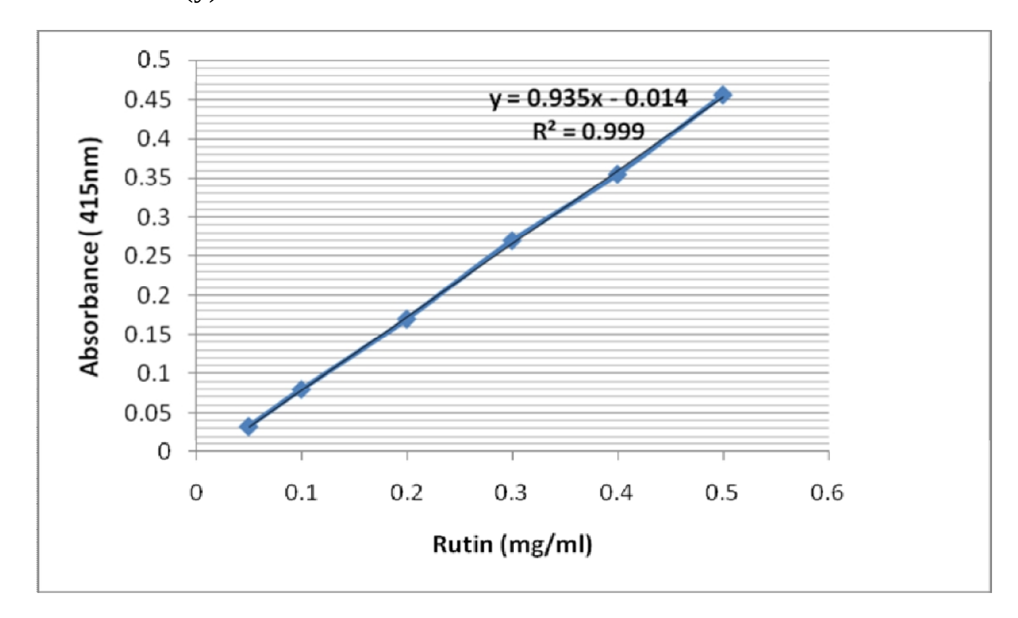


Figure (2): Calibration curve for the determination of total flavonoids contents in extracts, calculated by linear regression analysis. Concentrations of rutin (x) versus the absorbance (y).

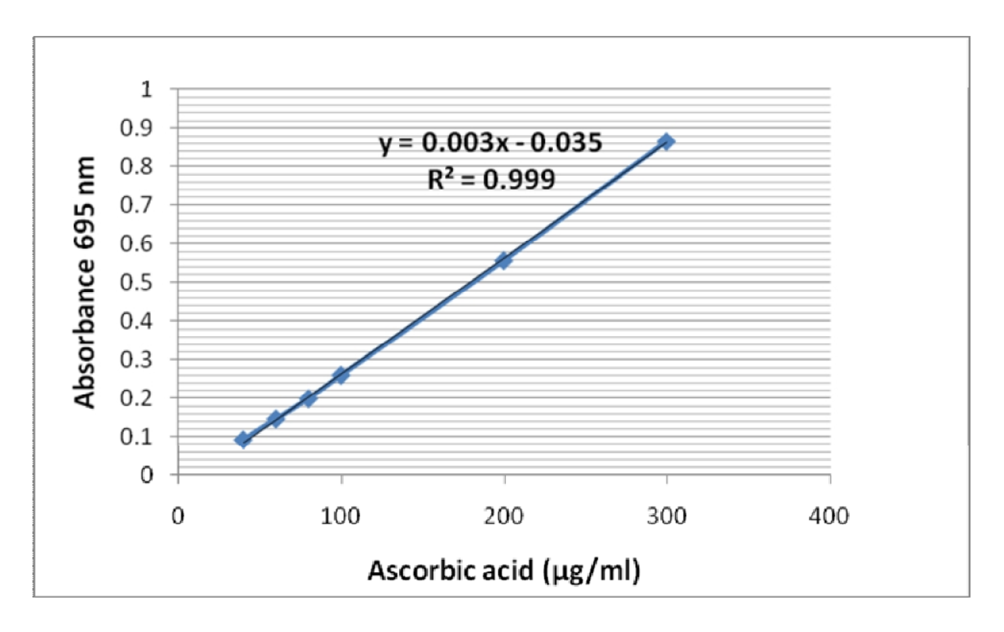


Figure (3): Calibration curve for the determination of total antioxidant capacity in extracts, calculated by linear regression analysis. Concentrations of ascorbic acid (x) versus the absorbance (y).

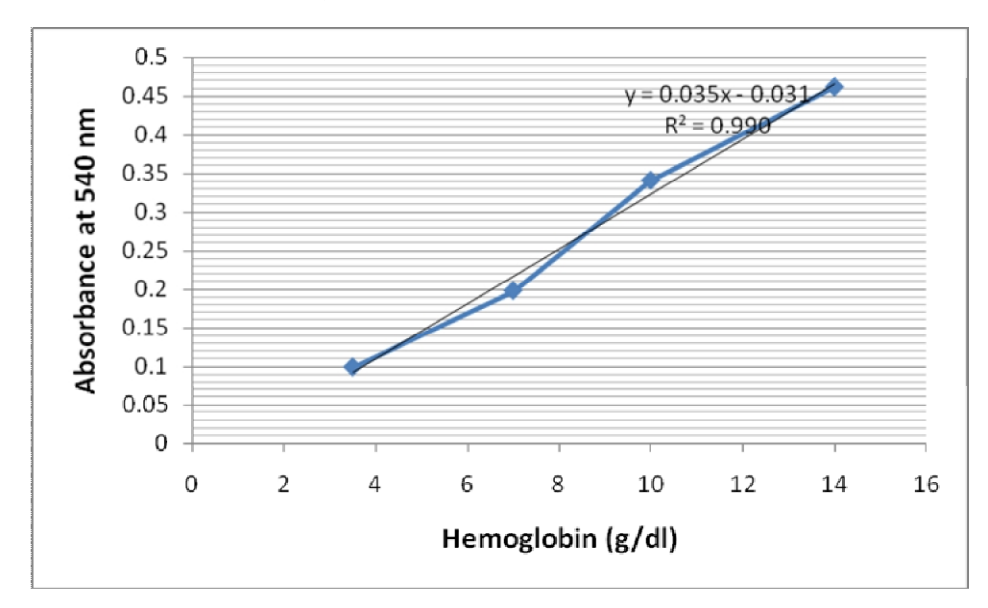


Figure (4): Calibration curve for the hemoglobin (g/dl) determination calculated by linear regression analysis. Concentrations of hemoglobin (x) versus the absorbance (y).

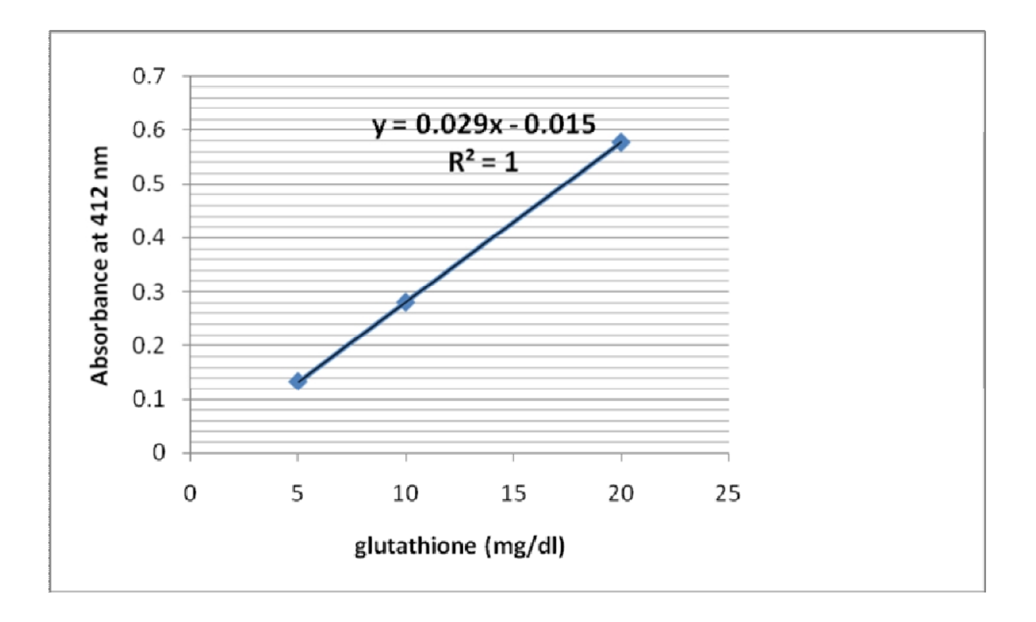


Figure (5): Calibration curve for the glutathione (mg/dl) determination calculated by linear regression analysis. Concentrations of glutathione (x) versus the absorbance (y).

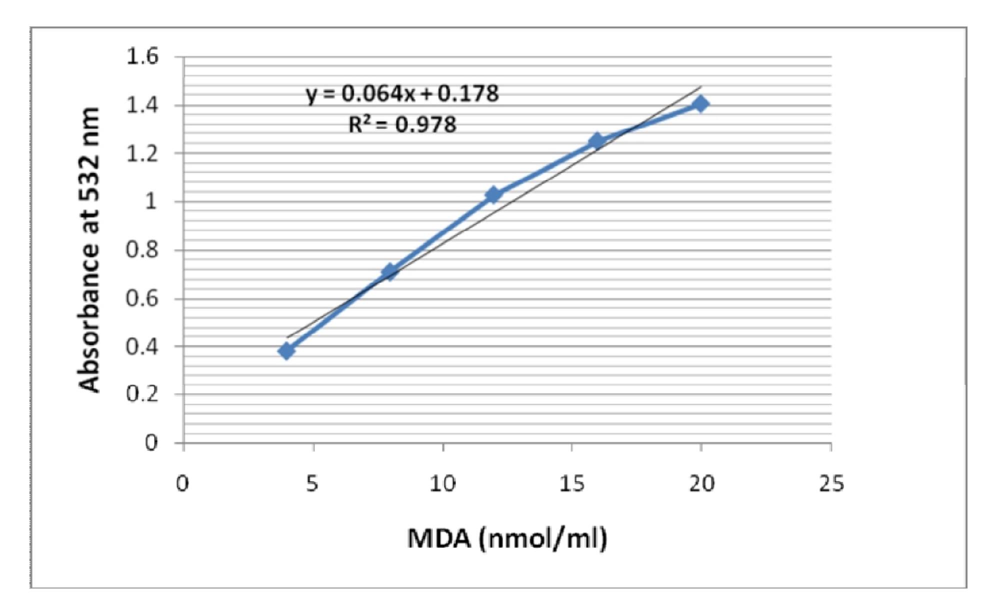


Figure (6): Calibration curve for the MDA (nmol/ml) determination calculated by linear regression analysis. Concentrations of MDA (x) versus the absorbance (y).

Table I: % of inhibition of DPPH free radical at different concentrations (10- 200 µg/ ml) of each plant extract.

Plant material	Concentration (µg/ ml)	% Inhibition mean ± S.D.n=3		*IC_{50} (µg/ ml)
Arbutus andrachne	10	38.0± 0.3	y=33,95x+9,888	15.2
	20	53.2± 0.6		
	50	76.4± 0.3		
	100	78.8±0.3		
	200	81.5±0.6		
Hypericumtriquetrifolium	10	23.5±0.4	y=43,38x-9,551	23.5
	20	46.8±0.5		
	50	74.3±0.2		
	100	77.8±0.2		
	200	81.2±0.5		
Pistacia palaestina	10	37±0.6	y=33,64x+10,52	14.9
	20	53.0±0.8		
	50	74.9±0.4		
	100	78.8±0.8		
	200	80.3±0.3		
Zingiber officinale	10	26.0±0.6	y=41,93x-17.4	40.4
	20	35.0±0.7		
	50	57.09±0.5		
	100	71.4±0.4		
	200	75.5.0±0.4		
Mentha Spp.	10	27.7±1.2	y=46,30x-15,66	26.2
	20	45.0±0.5		
	50	72.0±0.6		
	100	80.8±0.5		
	200	88.2±0.8		

Rosmarinus officinalis	10	26.9± 0.2	y=39,50x-8,569	30.4
	20	38.5±0.5		
	50	68.8±0.9		
	100	72.1±o.5		
	200	75.3±0.4		
Salvia triloba	10	27.7±1.2	y=47,87x-16,75	24.8
	20	45.0±0.5		
	50	71.9±0.5		
	100	80.5±0.5		
	200	88.2±0.7		
Verbena triphylla	10	27.2±0.2	y=40,64x-11,33	32.3
	20	36.7±0.6		
	\50	69.6±0.5		
	100	72.1±05		
	200	75.3±0.3		
Origanum syriacum	10	9.2±0.7	y=55,55x-47,01	55.8
	20	23.4±0.5		
	50	46.4±0.4		
	100	69.1±0.3		
	200	77.9±0.8		
Teucrium polium	10	9.2± 0.5	y=55,76x-55,83	79.0
	20	13.6± 0.2		
	50	31.0± 0.4		
	100	64.8±0.4		
	200	73.5±0.6		
***Nigella sativum*, ethanolic**	10	12±0.3	y=12,80x+3,326	4043
	20	12.7±0.2		
	50	16.4±0.3		
	100	21.2±0.3		
	200	28.7±0.5		

Ceratonia siliqua	10	1.2±0.1	y=19,18x-2!,51	5350
	20	1.9± 0.15		
	50	2.9±0.4		
	100	9.9± 0.9		
	200	20.0±0.6		
***Nigella sativum*, methanolic**	10	10.4±0.2	y=7,377x+2,077	3013
	20	12.0±0.1		
	50	13.5±0.1		
	100	15.3±0.2		
	200	21.0±0.1		

*****IC_{50} was calculated by using microsoft excel: The concentration values were transformed into log, then a scatter graph was constructed using excel with y-axis=% inhibition and x-axis= log conc. Finally a trendline was added to obtain linear equation y=mx + c. From this equation, y was substituted by 50 to get x=log conc. The antilog was then calculated.

Table II: % of inhibition of iron chelating activity at different concentrations (10- 200 µg/ml) of plant extracts.

Plant material	Concentration (µg/ ml)	% Inhibition mean ± S.D.n=3		*IC_{50} (µg/ ml)
Nigella sativum/methanol	10	2.3± 0.3	Y=64,43X-71,13	75
	50	21.9±0.5		
	100	61.6±0.5		
	200	80.7±1.5		
Nigella sativum/ethanol	10	2.6± 0.5	Y=63,67X-70,03	77
	50	22±0.7		
	100	61.6±0.5		
	200	80.5±1.3		
Pistacia palaestina	10	17.9± 0.3	Y=38,29X-24,40	87.7
	50	28.5±0.5		
	100	58.9±0.3		
	200	65.0±0.3		
Arbutus andrachne	10	11.3± 0.7	Y=44,43X-38,14	96.3
	50	23.1±1.2		
	100	57.6±1.5		
	200	66.4±0.8		
Hypericumtriquetrifolium	10	10.6± 0.7	Y=36,83X-31,39	162.1
	50	19.4±1.0		
	100	30.8±1.0		
	200	31.2±0.6		
Mentha Spp.	10	1.6± 0.4	Y=24,33X-21,83	896.0
	50	19.4±1.0		
	100	30.8±1.0		
	200	31.2±0.6		
Teucrium polium	10	1.6± 0.4	Y=35,94X-39,22	304
	50	10.5±0.3		
	100	41.2±1.0		
	200	43.6±0.5		

Origanum syriacum	10	2.7±0.3	Y=24,03X-22,29	1002
	50	15.8±0.8		
	100	27.1±1.2		
	200	33.4±0.8		
Salvia triloba	10	3..3±0.5	Y=16,75X-12,24	5019
	50	17.7±0.4		
	100	23.7±0.6		
	200	23.7±0.6		
Zingiber officinale	10	0.0± 0.0	Y=22,94X-29,69	2095
	50	0.0± 0.0		
	100	5.6±0.5		
	200	36.2±0.3		
Verbena triphylla	10	0.0± 0.0	Y=25,25X-30,91	1601
	50	1.6± 0.3		
	100	15.9±0.8		
	200	35.6±0.5		
Rosmarinus officinalis	10	0.0± 0.0	Y=16,95X-19,66	12087
	50	2±0.7		
	100	16.8±1.0		
	200	21.2±0.8		

*****IC50 was calculated by using microsoft excel: The concentration values were transformed into log, then a scatter graph was constructed using excel with y-axis=% inhibition and x-axis= log conc. Finally a trendline was added to obtain linear equation y=mx + c. From this equation, y was substituted by 50 to get x=log conc. The antilog was then calculated.

- **Phosphate Buffered Saline (PBS):**

A stock solution of PBS (75mmol/l phosphate, 50% normal saline) was prepared by mixing: 1.8 parts of 150 mmol/l NaH_2PO_4, 8.2 parts of 150 mmol/l Na_2HPO_4 and 10 parts of normal saline. The pH of this solution was adjusted to 7.40 ± 0.02 using NaOH/HCl.

- **Drabkin's Reagent:**

The following reagents: 200 mg of potassium ferricyanide, 50 mg of potassium cyanide and 140 mg of potassium dihydrogen phosphate were dissolved in 1000 ml of distilled water and mixed. This solution was stored at room temperature in a brown glass bottle.

- **Precipitating Solution:**

The following reagents: 1.67 g of glacial metaphosphoric acid, 0.2 g of disodium–EDTA and 30 g of sodium chloride were dissolved in 100 ml of distilled water and mixed and heated.

Appendix II

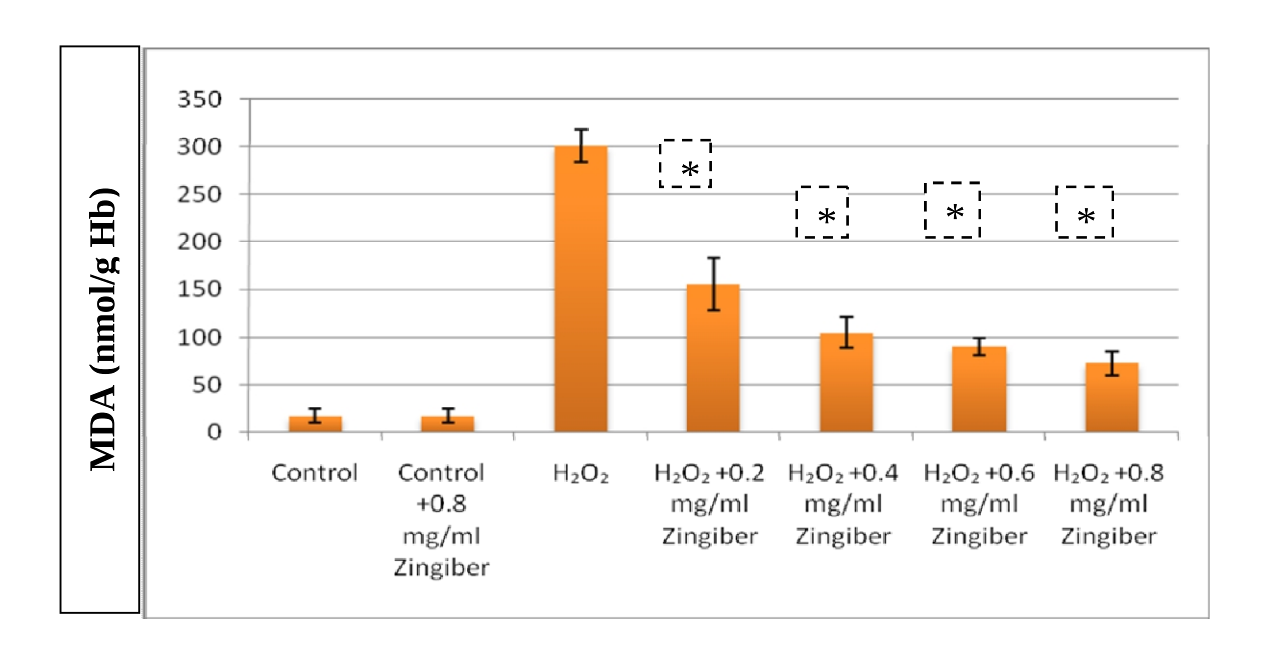


Figure 1: MDA concentration of normal erythrocytes when incubated at 37°C for 60 min in the absence or in the presence of 10 mM H_2O_2, or in the presence of 10 mM H_2O_2 plus different concentrations of *Zingiber officinale* methanolic extract. Each column represents the mean value and bars represent the S.D. (n = 7).*P< 0.05, compared to treatment with H_2O_2 alone.

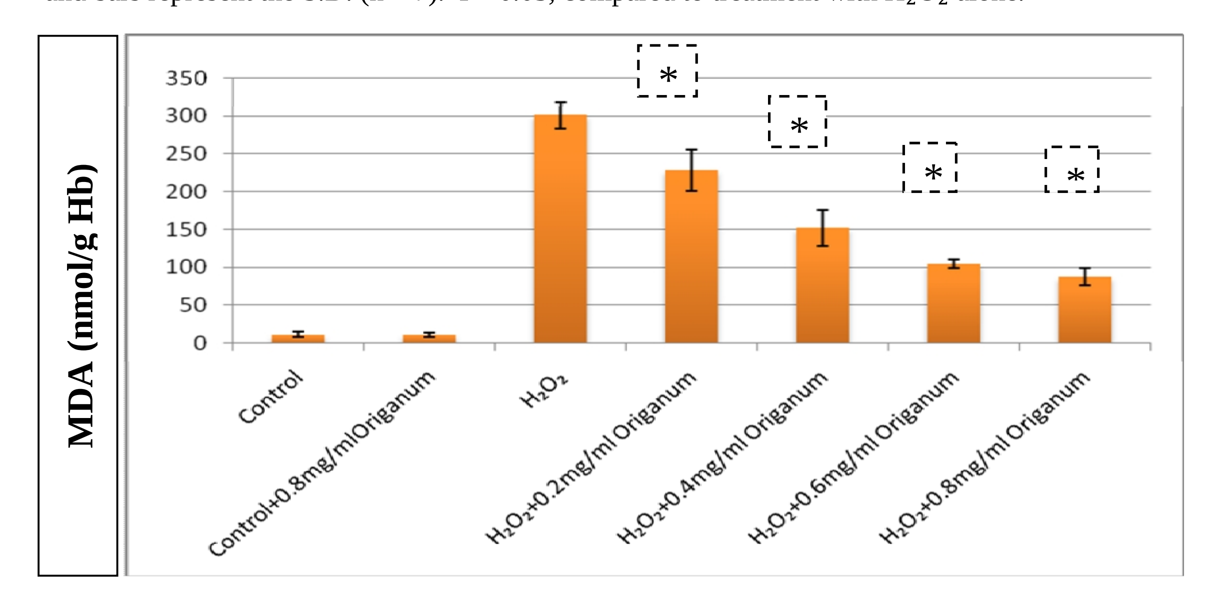


Figure 2: MDA concentration of normal erythrocytes when incubated at 37°C for 60 min in the absence or in the presence of 10 mM H_2O_2, or in the presence of 10 mM H_2O_2 plus different concentrations of *Origanum syriacum* methanolic extract. Each column represents the mean value and bars represent the S.D. (n = 7).*P< 0.05, compared to treatment with H_2O_2 alone.

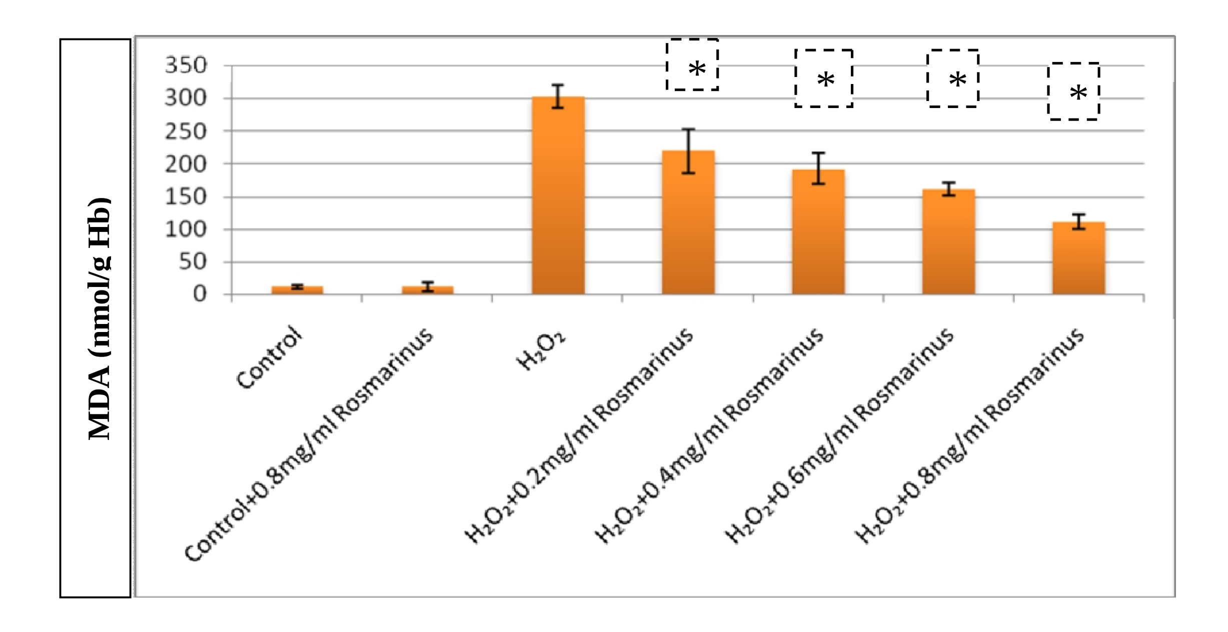


Figure 3: MDA concentration of normal erythrocytes when incubated at 37°C for 60 min in the absence or in the presence of 10 mM H_2O_2, or in the presence of 10 mM H_2O_2 plus different concentrations of *Rosmarinus officinalis* methanolic extract. Each column represents the mean value and bars represent the S.D. (n = 7).*P< 0.05, compared to treatment with H_2O_2 alone.

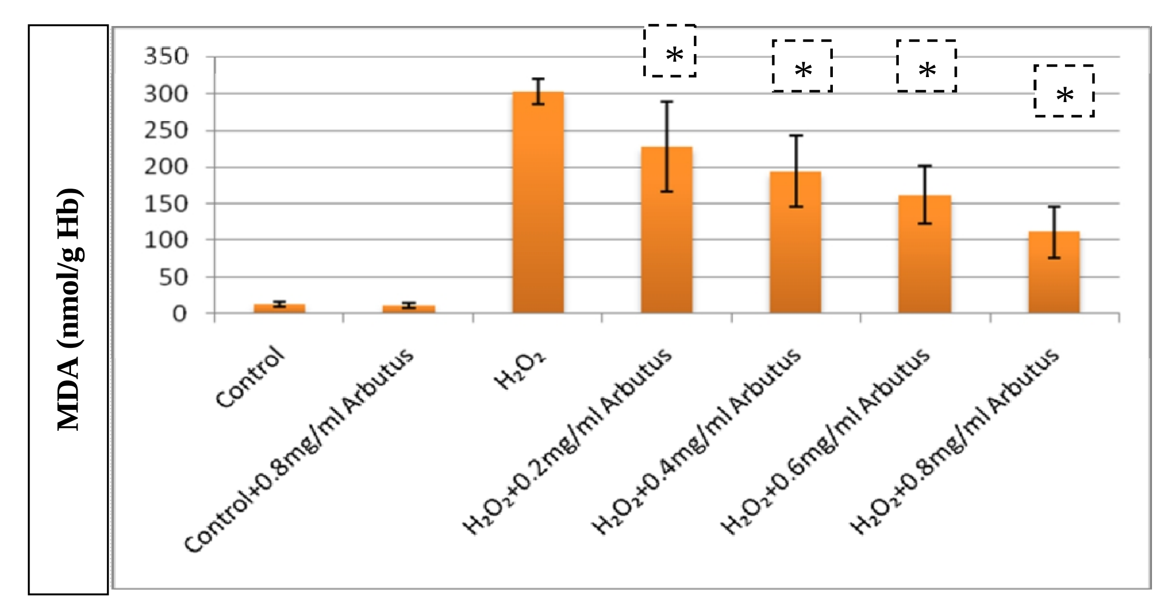


Figure 4: MDA concentration of normal erythrocytes when incubated at 37°C for 60 min in the absence or in the presence of 10 mM H_2O_2, or in the presence of 10 mM H_2O_2 plus different concentrations of *Arbutus andrachne* methanolic extract. Each column represents the mean value and bars represent the S.D. (n = 7).*P< 0.05, compared to treatment with H_2O_2 alone.

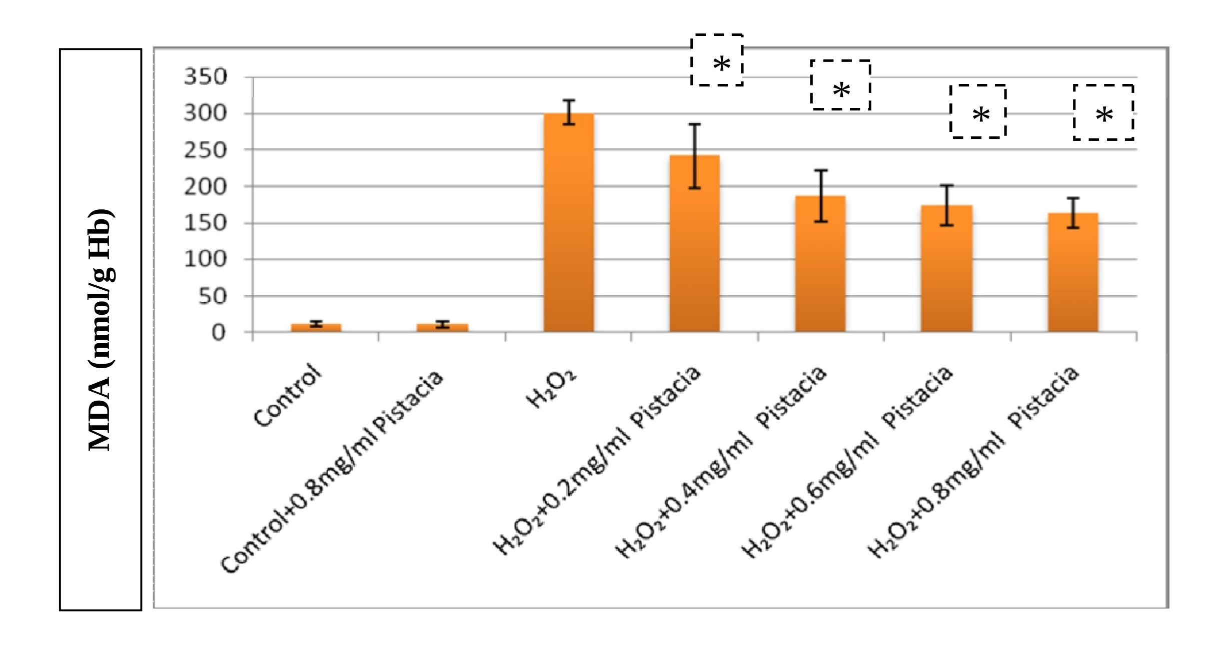


Figure 5: MDA concentration of normal erythrocytes when incubated at 37°C for 60 min in the absence or in the presence of 10 mM H_2O_2, or in the presence of 10 mM H_2O_2 plus different concentrations of *Pistacia palaestina* methanolic extract. Each column represents the mean value and bars represent the S.D. (n = 7).*P< 0.05, compared to treatment with H_2O_2 alone.

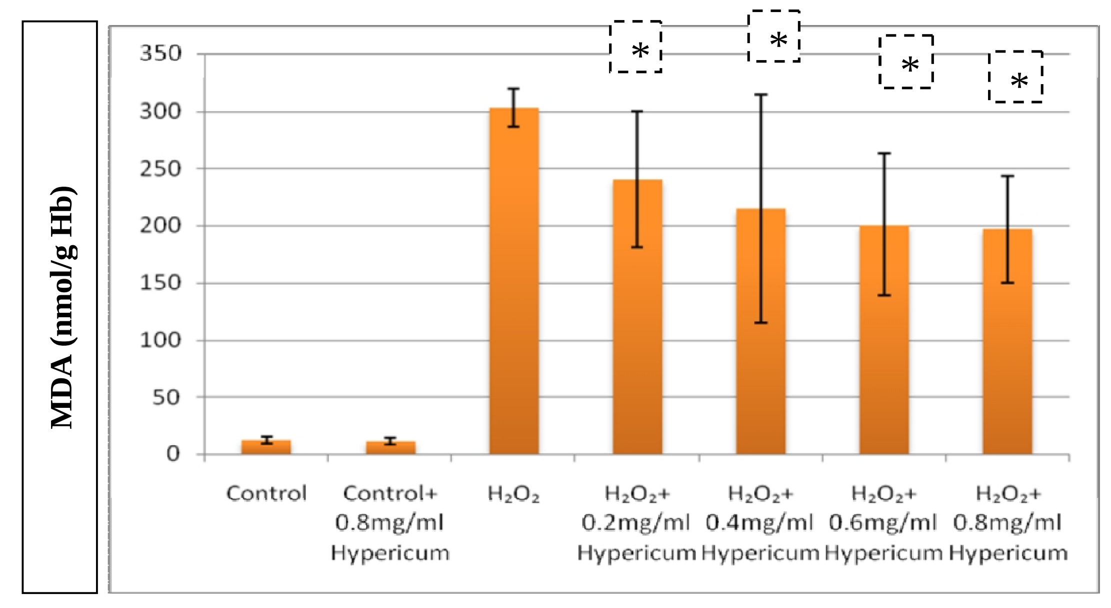


Figure 6: MDA concentration of normal erythrocytes when incubated at 37°C for 60 min in the absence or in the presence of 10 mM H_2O_2, or in the presence of 10 mM H_2O_2 plus different concentrations of *Hypericum triquetrifolium* methanolic extract. Each column represents the mean value and bars represent the S.D. (n = 7).*P< 0.05, compared to treatment with H_2O_2 alone.

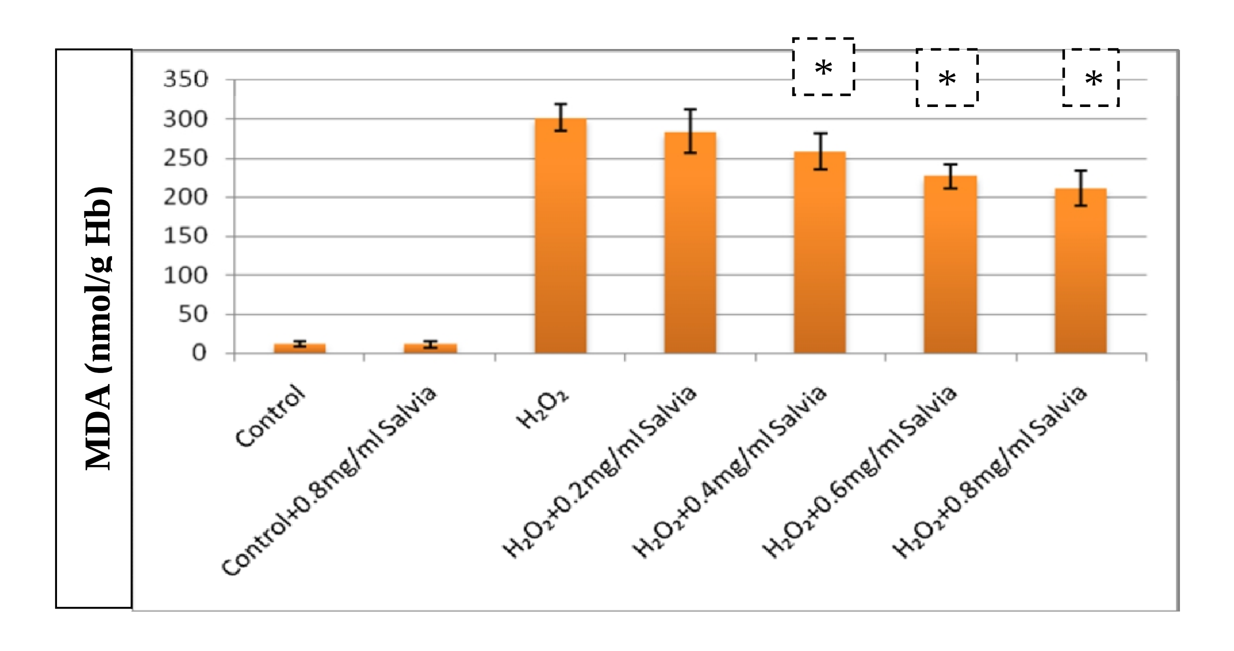


Figure 7: MDA concentration of normal erythrocytes when incubated at 37°C for 60 min in the absence or in the presence of 10 mM H_2O_2, or in the presence of 10 mM H_2O_2 plus different concentrations of *Salvia triloba* methanolic extract. Each column represents the mean value and bars represent the S.D. (n = 7).*P< 0.05, compared to treatment with H_2O_2 alone.

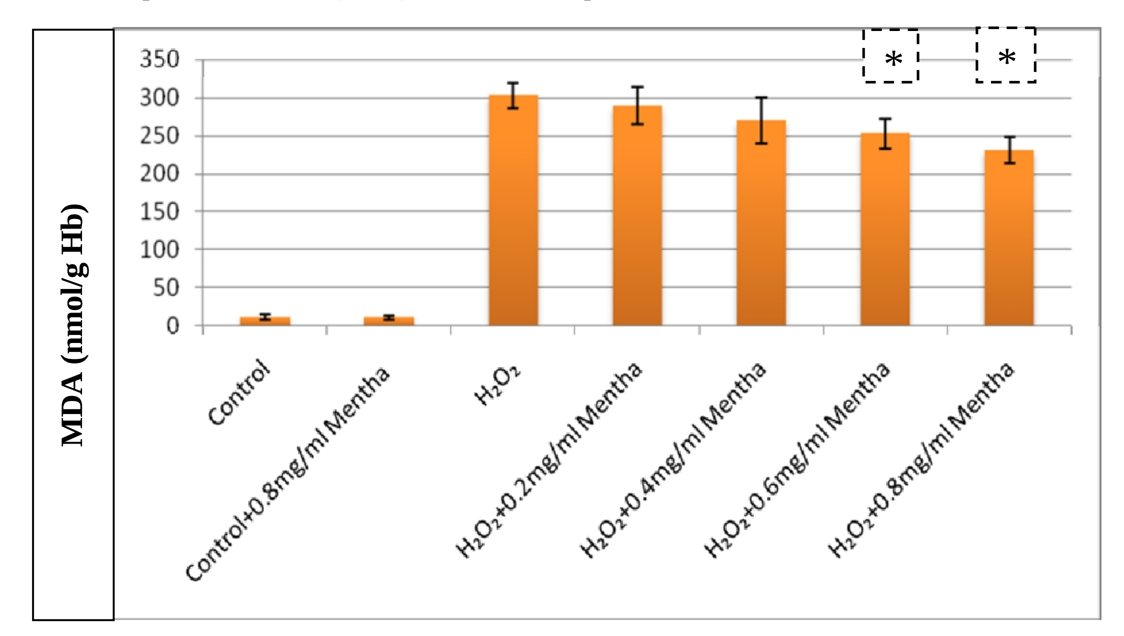


Figure 9: MDA concentration of normal erythrocytes when incubated at 37°C for 60 min in the absence or in the presence of 10 mM H_2O_2, or in the presence of 10 mM H_2O_2 plus different concentrations of *Mentha Spp.* methanolic extract. Each column represents the mean value and bars represent the S.D. (n = 7).*P< 0.05, compared to treatment with H_2O_2 alone.

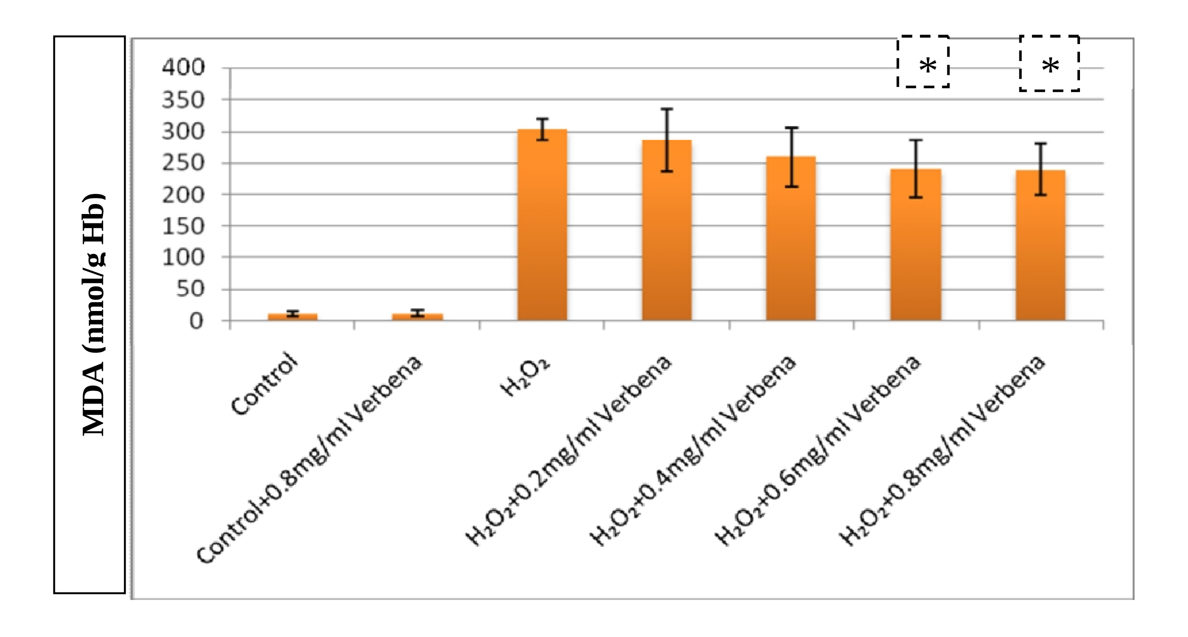


Figure 8: MDA concentration of normal erythrocytes when incubated at 37°C for 60 min in the absence or in the presence of 10 mM H_2O_2, or in the presence of 10 mM H_2O_2 plus different concentrations of *Verbena triphylla* methanolic extract. Each column represents the mean value and bars represent the S.D. (n = 7).*P< 0.05, compared to treatment with H_2O_2 alone.

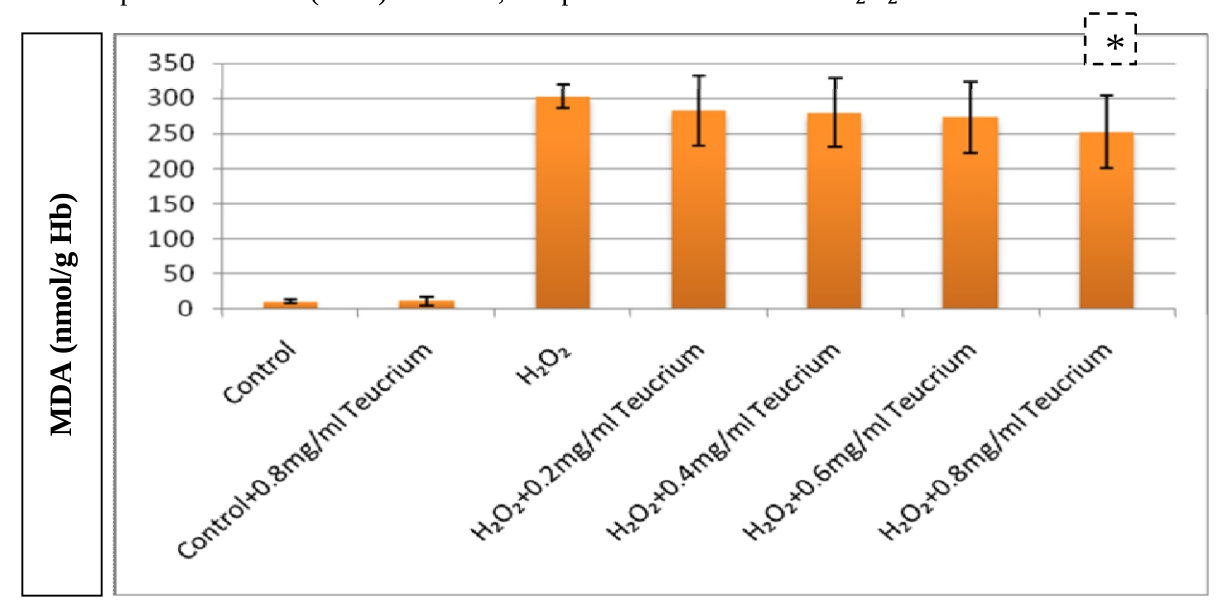


Figure 10: MDA concentration of normal erythrocytes when incubated at 37°C for 60 min in the absence or in the presence of 10 mM H_2O_2, or in the presence of 10 mM H_2O_2 plus different concentrations of *Teucrium polium* methanolic extract. Each column represents the mean value and bars represent the S.D. (n = 7).*P< 0.05, compared to treatment with H_2O_2 alone.

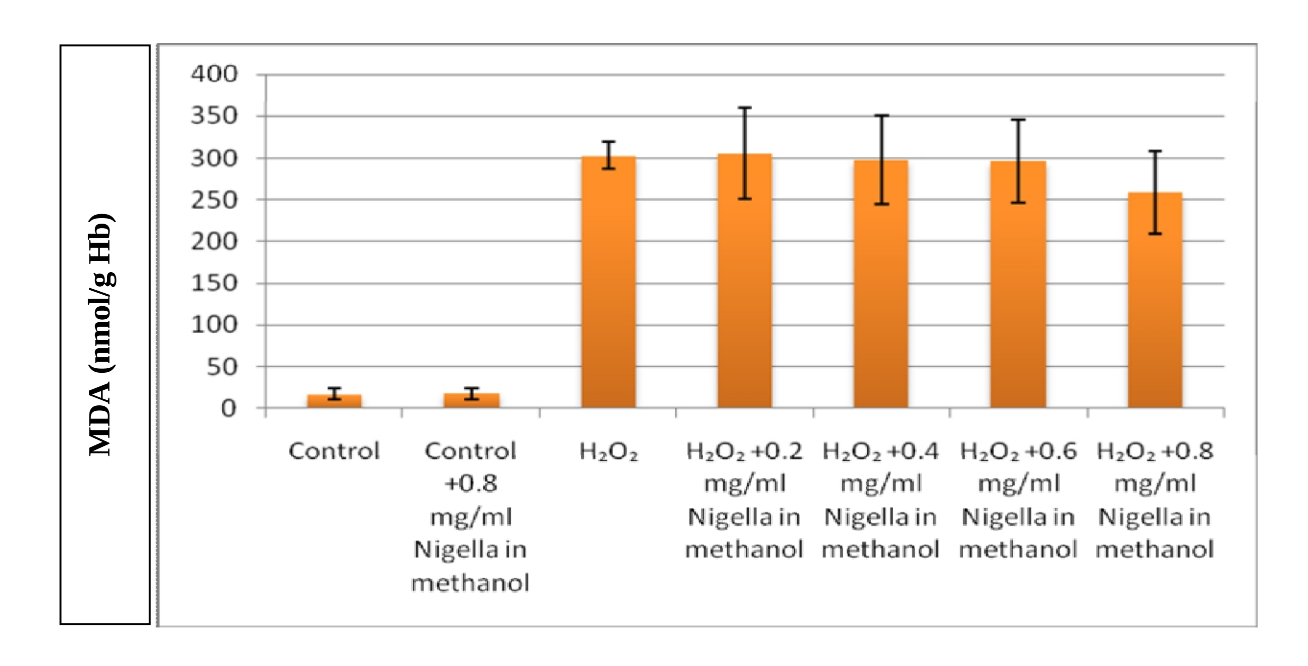


Figure 11: MDA concentration of normal erythrocytes when incubated at 37°C for 60 min in the absence or in the presence of 10 mM H_2O_2, or in the presence of 10 mM H_2O_2 plus different concentrations of *Nigella sativum* methanolic extract. Each column represents the mean value and bars represent the S.D. (n = 7).

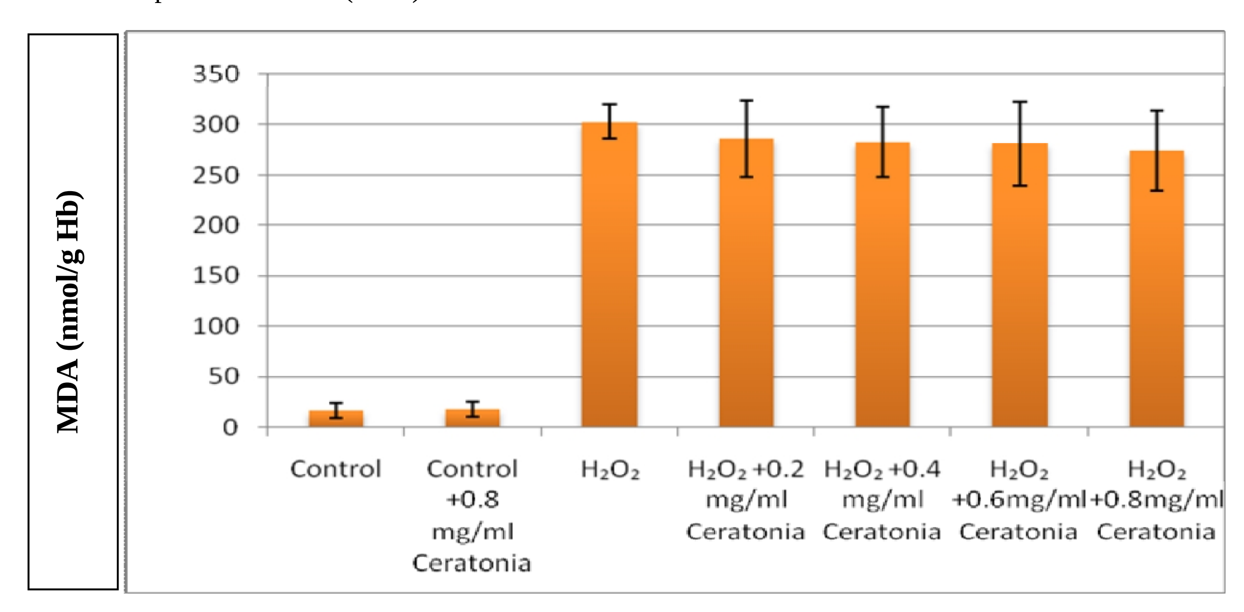


Figure 12: MDA concentration of normal erythrocytes when incubated at 37°C for 60 min in the absence or in the presence of 10 mM H_2O_2, or in the presence of 10 mM H_2O_2 plus different concentrations of *Ceratonia siliqua* methanolic extract. Each column represents the mean value and bars represent the S.D. (n = 7).

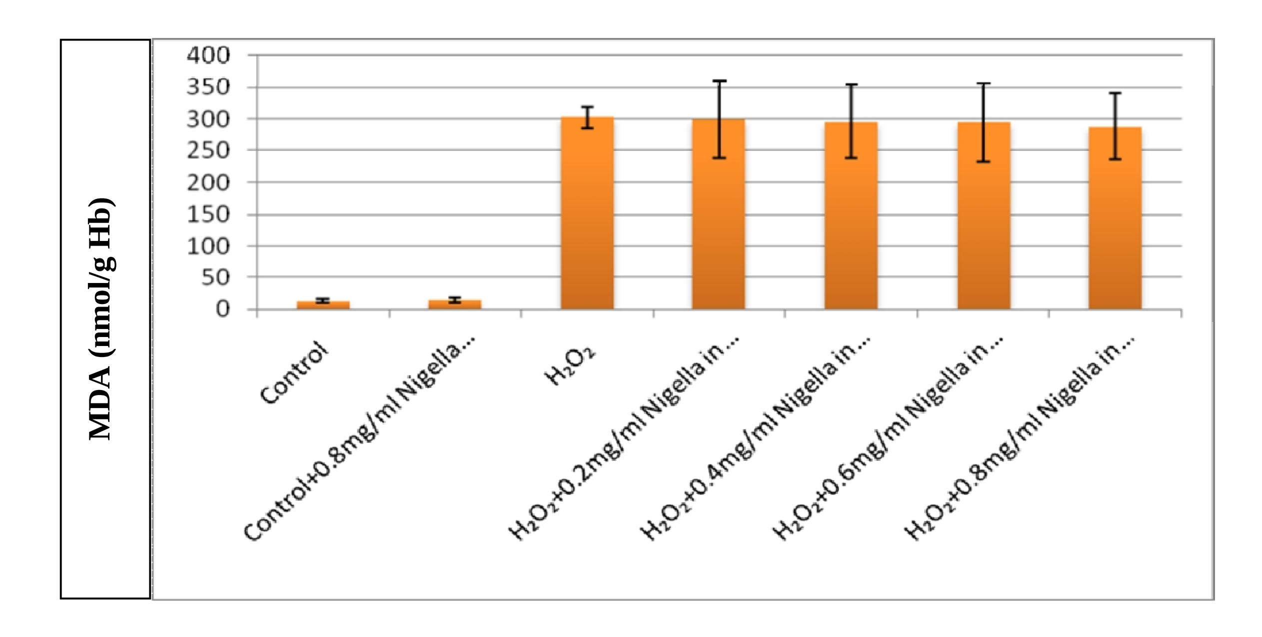


Figure 13: MDA concentration of normal erythrocytes when incubated at 37°C for 60 min in the absence or in the presence of 10 mM H_2O_2, or in the presence of 10 mM H_2O_2 plus different concentrations of *Nigella sativum* ethanolic extract. Each column represents the mean value and bars represent the S.D. (n = 7).

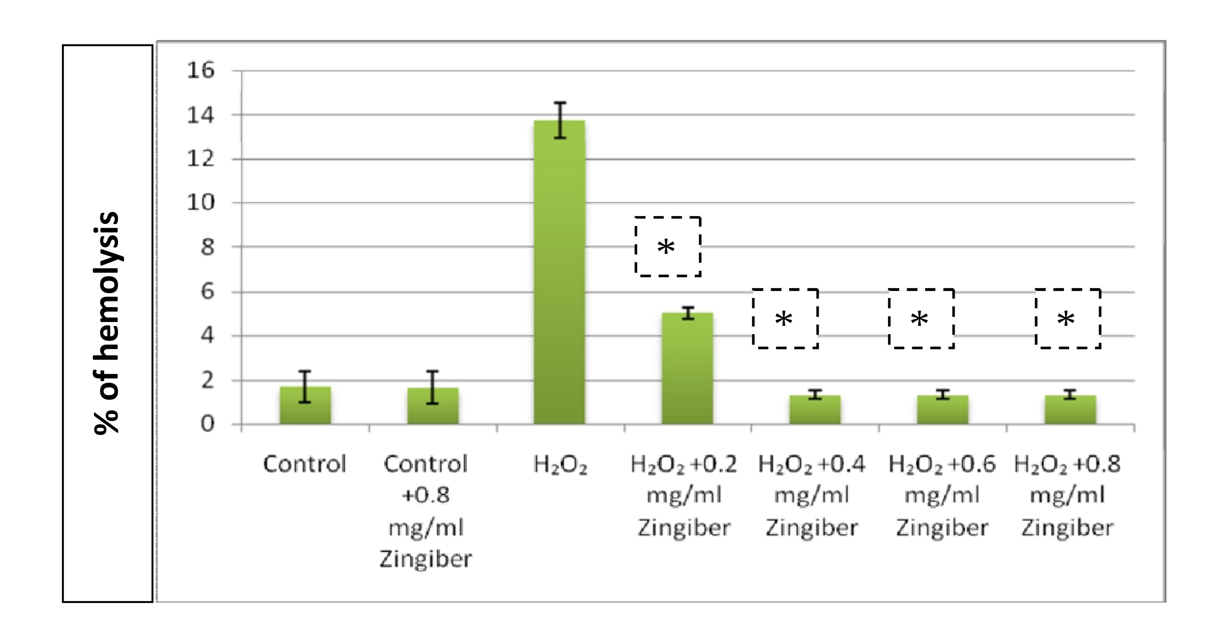


Figure 14: Percentage hemolysis of normal erythrocytes when incubated at 37°C for 60 min in the absence or in the presence of 10 mM H_2O_2, or in the presence of 10 mM H_2O_2 plus different concentrations of *Zingiber officinale* methanolic extract. Each column represents the mean value and bars represent the S.D. (n = 7).*P< 0.05, compared to treatment with H_2O_2 alone.

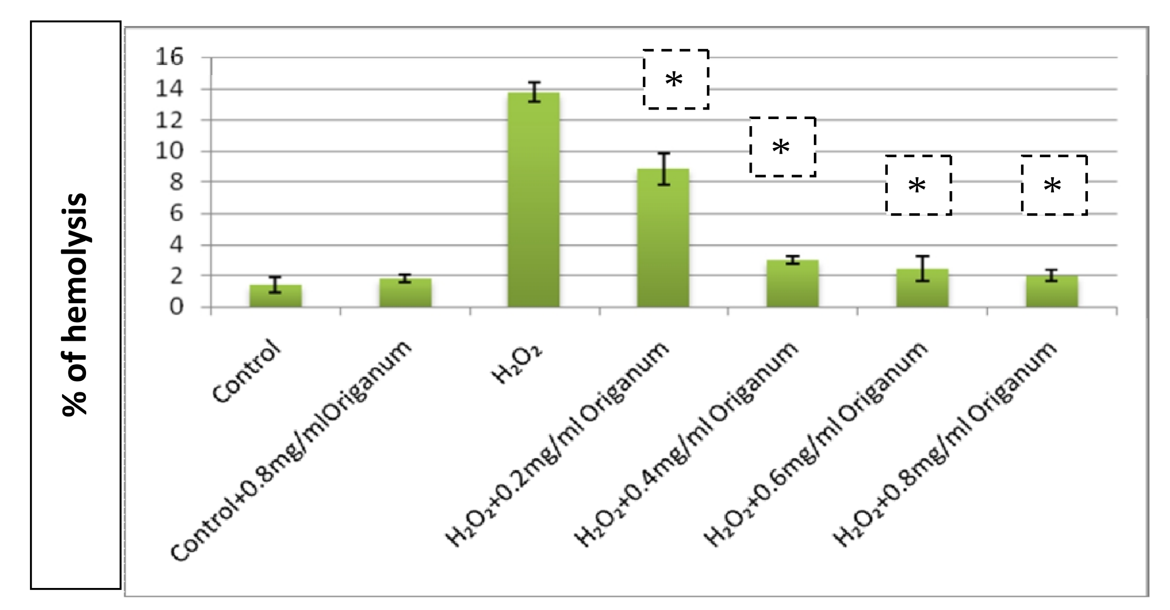


Figure 15: Percentage hemolysis of normal erythrocytes when incubated at 37°C for 60 min in the absence or in the presence of 10 mM H_2O_2, or in the presence of 10 mM H_2O_2 plus different concentrations of *Origanum syriacum* methanolic extract. Each column represents the meanvalue and bars represent the S.D. (n = 7).*P< 0.05, compared to treatment with H_2O_2 alone.

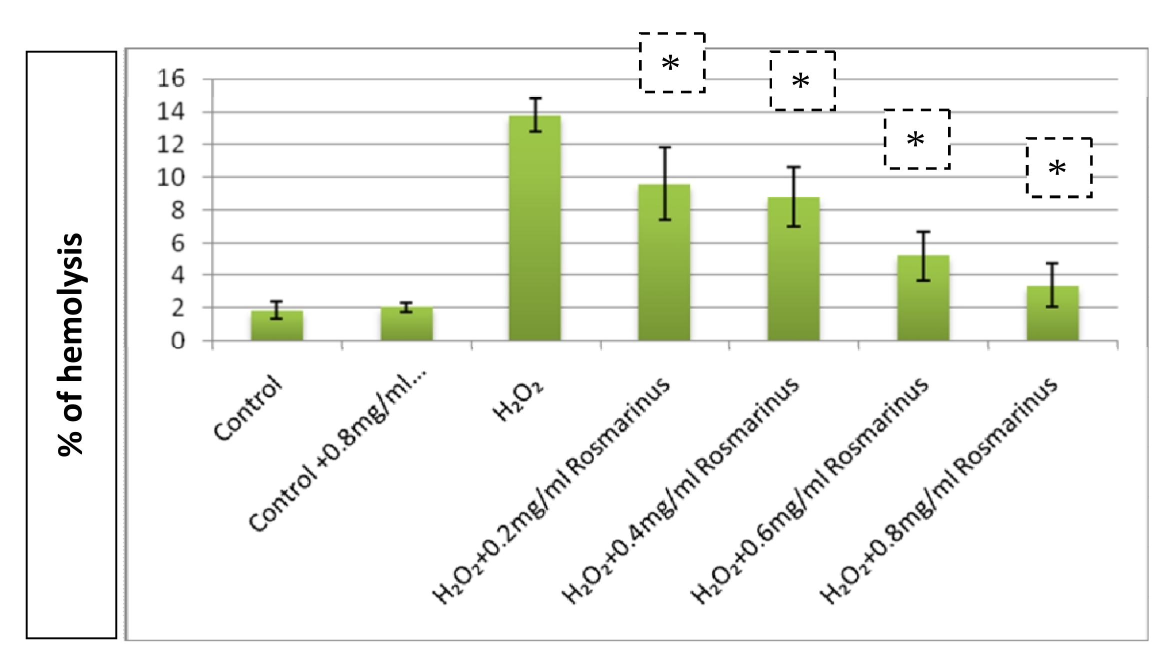


Figure 16: Percentage hemolysis of normal erythrocytes when incubated at 37°C for 60 min in the absence or in the presence of 10 mM H_2O_2, or in the presence of 10 mM H_2O_2 plus different concentrations of *Rosmarinus officinalis* methanolic extract. Each column represents the mean value and bars represent the S.D. (n = 7).*P< 0.05, compared to treatment with H_2O_2 alone.

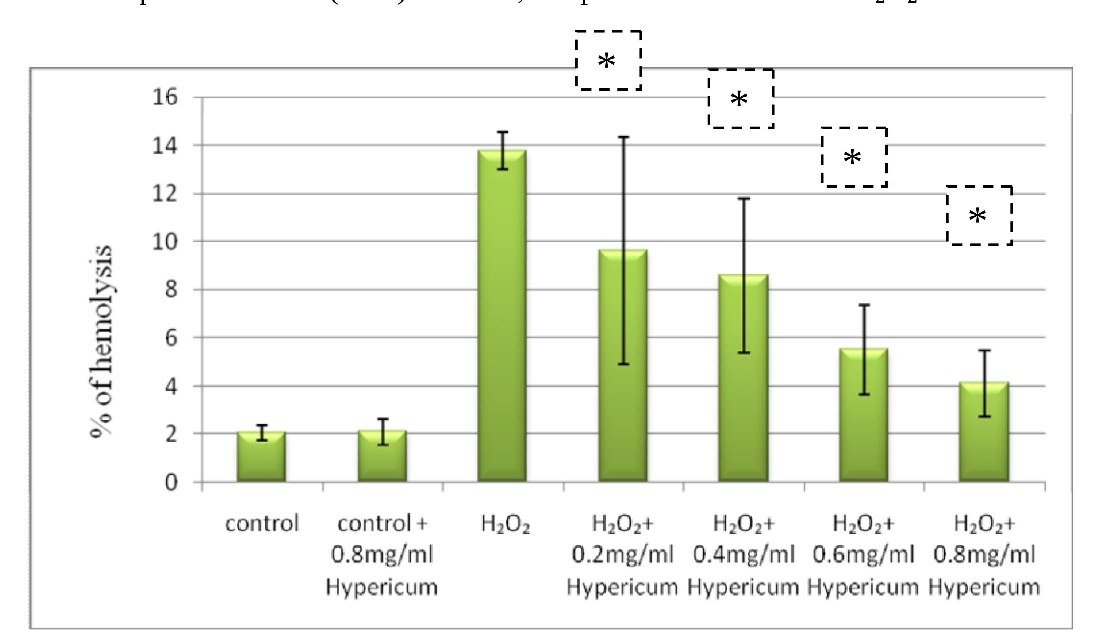


Figure 17: Percentage hemolysis of normal erythrocytes when incubated at 37°C for 60 min in the absence or in the presence of 10 mM H_2O_2, or in the presence of 10 mM H_2O_2 plus different concentrations of *Hypericum triquetrifolium* methanolic extract. Each column represents the mean value and bars represent the S.D. (n = 7).*P< 0.05, compared to treatment with H_2O_2 alone.

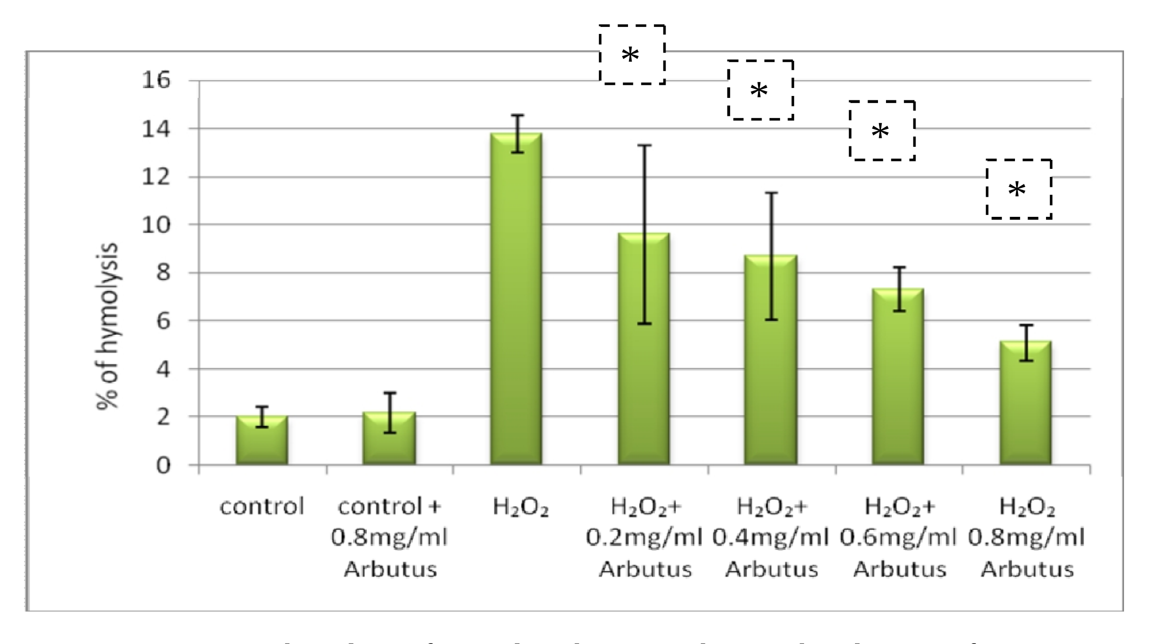


Figure 18: Percentage hemolysis of normal erythrocytes when incubated at 37°C for 60 min in the absence or in the presence of 10 mM H_2O_2, or in the presence of 10 mM H_2O_2 plus different concentrations of *Arbutus andrachne* methanolic extract. Each column represents the mean value and bars represent the S.D. (n = 7).*P< 0.05, compared to treatment with H_2O_2 alone.

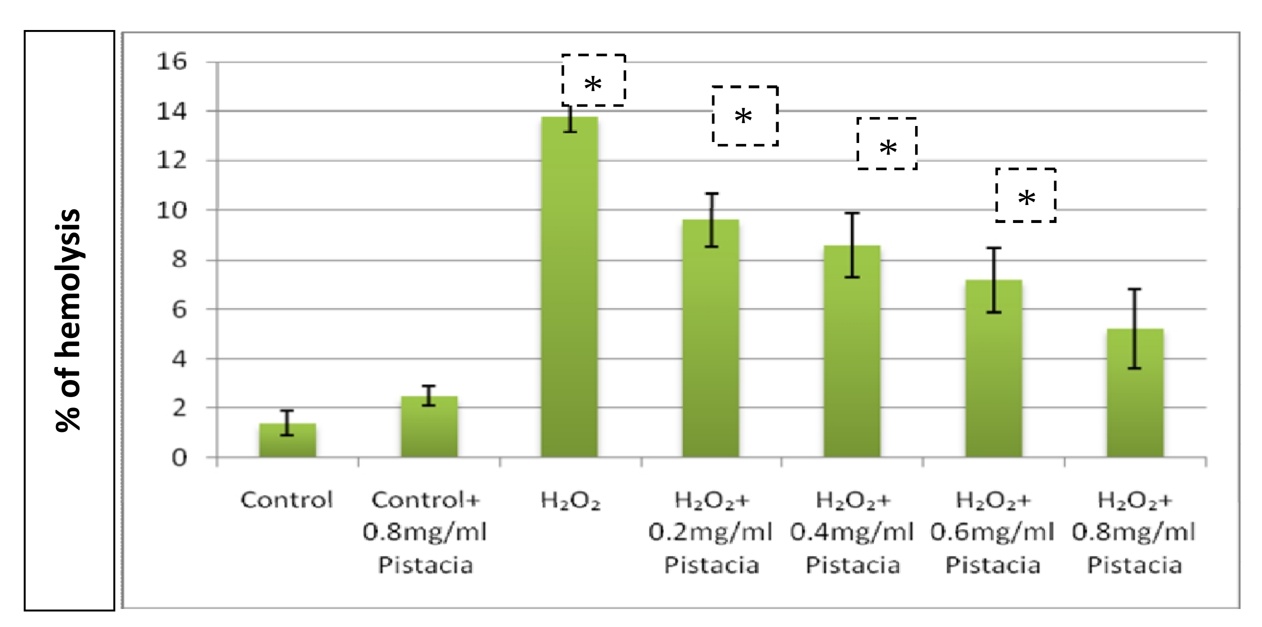


Figure 19: Percentage hemolysis of normal erythrocytes when incubated at 37°C for 60 min in the absence or in the presence of 10 mM H_2O_2, or in the presence of 10 mM H_2O_2 plus different concentrations of *Pistacia palaestina* methanolic extract. Each column represents the mean value and bars represent the S.D. (n = 7).*P< 0.05, compared to treatment with H_2O_2 alone.

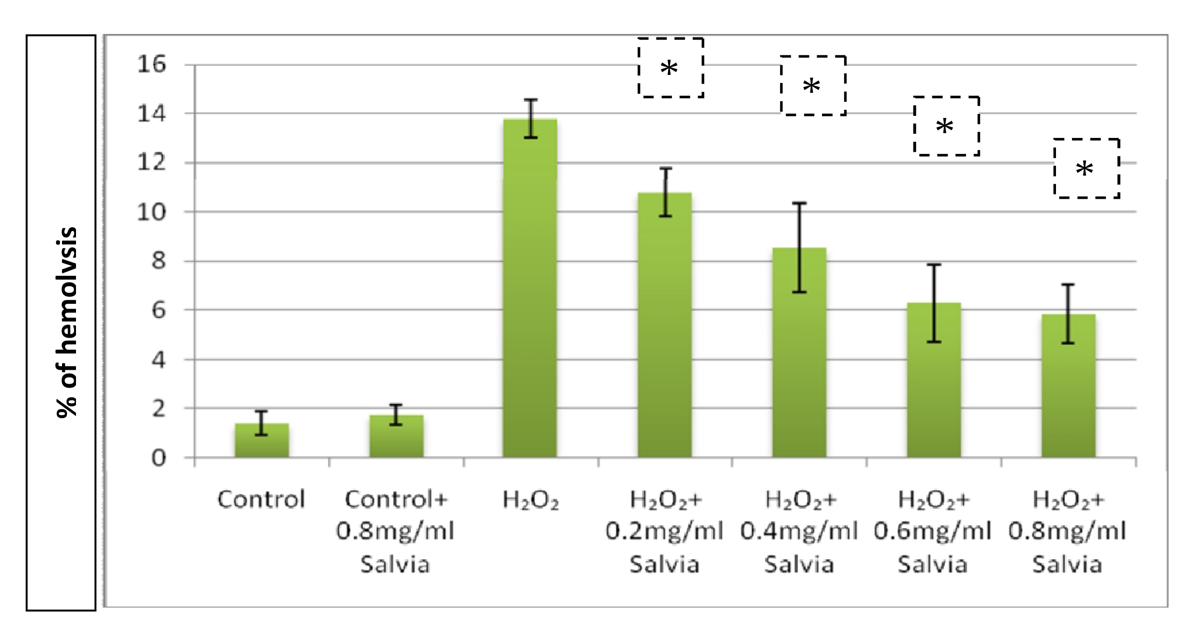


Figure 20: Percentage hemolysis of normal erythrocytes when incubated at 37°C for 60 min in the absence or in the presence of 10 mM H_2O_2, or in the presence of 10 mM H_2O_2 plus different concentrations of *Salvia triloba* methanolic extract. Each column represents the mean value and bars represent the S.D. (n = 7).*P< 0.05, compared to treatment with H_2O_2 alone.

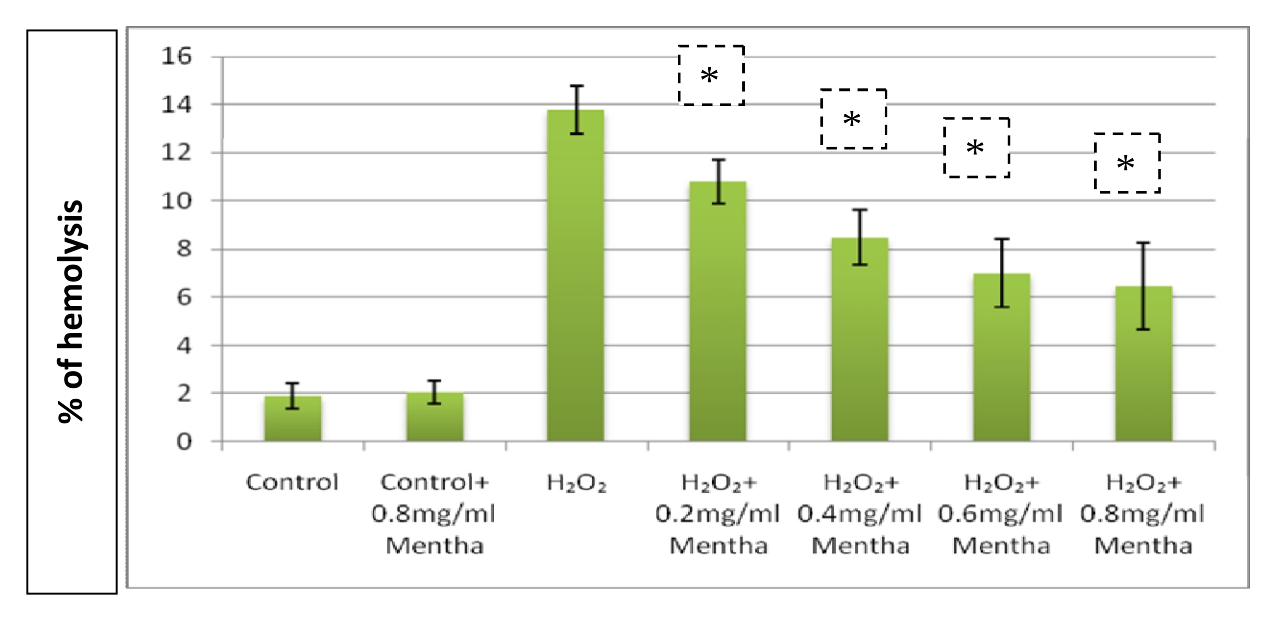


Figure 21: Percentage hemolysis of normal erythrocytes when incubated at 37°C for 60 min in the absence or in the presence of 10 mM H_2O_2, or in the presence of 10 mM H_2O_2 plus different concentrations of *Mentha Spp.* methanolic extract. Each column represents the mean value and bars represent the S.D. (n = 7).*P< 0.05, compared to treatment with H_2O_2 alone.

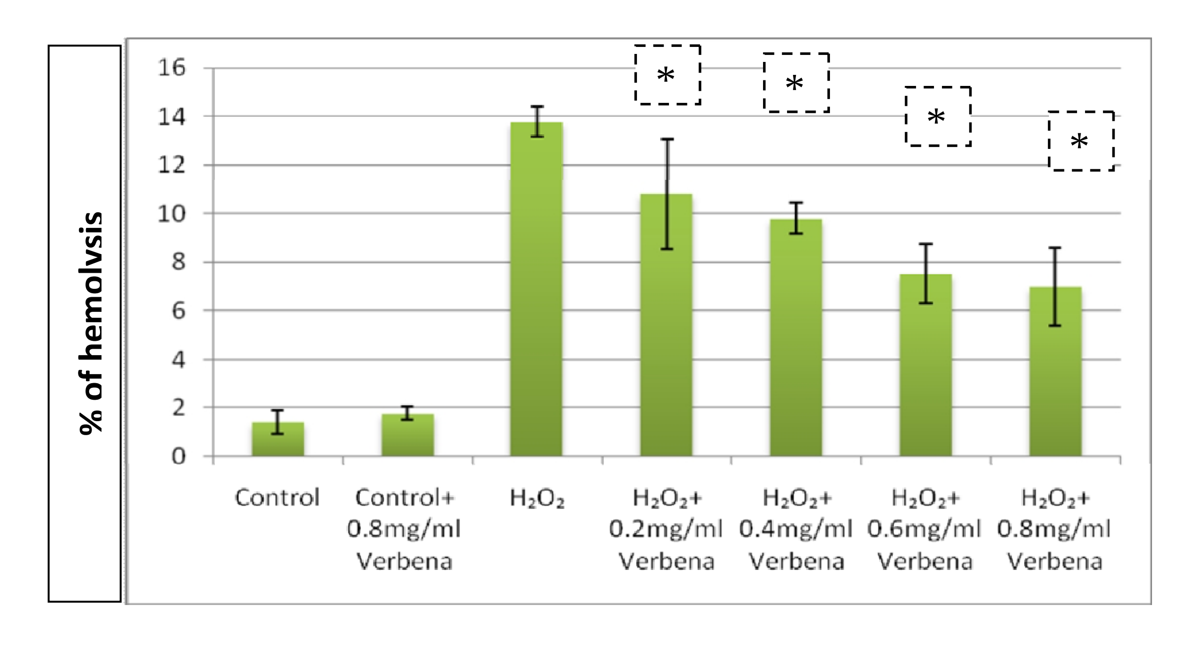


Figure 22: Percentage hemolysis of normal erythrocytes when incubated at 37°C for 60 min in the absence or in the presence of 10 mM H_2O_2, or in the presence of 10 mM H_2O_2 plus different concentrations of *Verbena triphylla* methanolic extract. Each column represents the mean value and bars represent the S.D. (n = 7).*P< 0.05, compared to treatment with H_2O_2 alone.

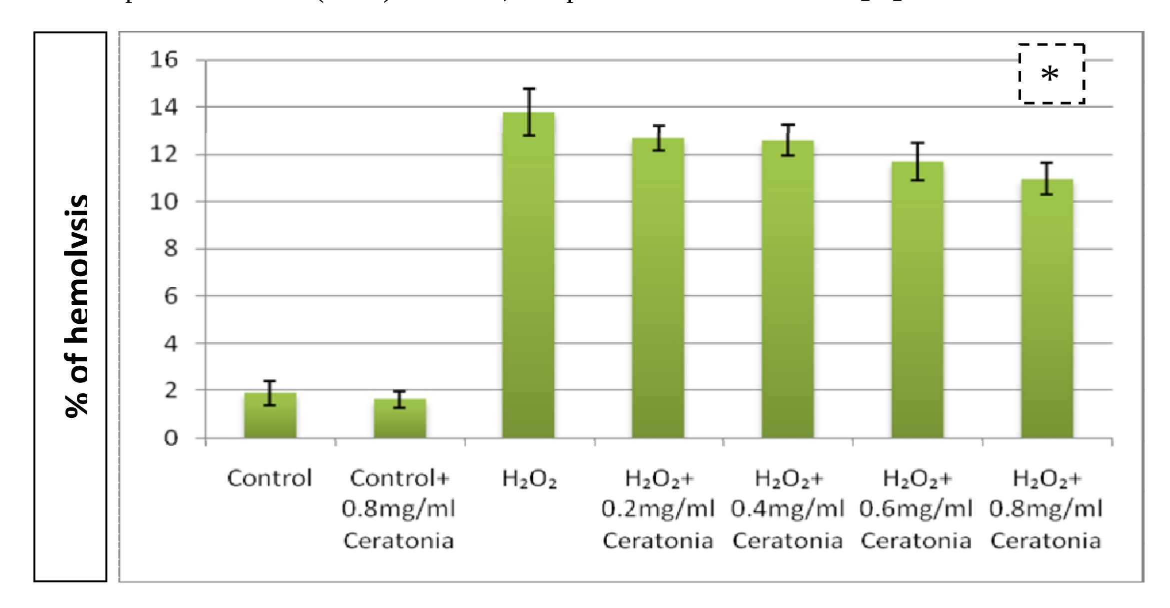


Figure 23: Percentage hemolysis of normal erythrocytes when incubated at 37°C for 60 min in the absence or in the presence of 10 mM H_2O_2, or in the presence of 10 mM H_2O_2 plus different concentrations of *Ceratonia siliqua* methanolic extract. Each column represents the mean value and bars represent the S.D. (n = 7). *P< 0.05, compared to treatment with H_2O_2 alone.

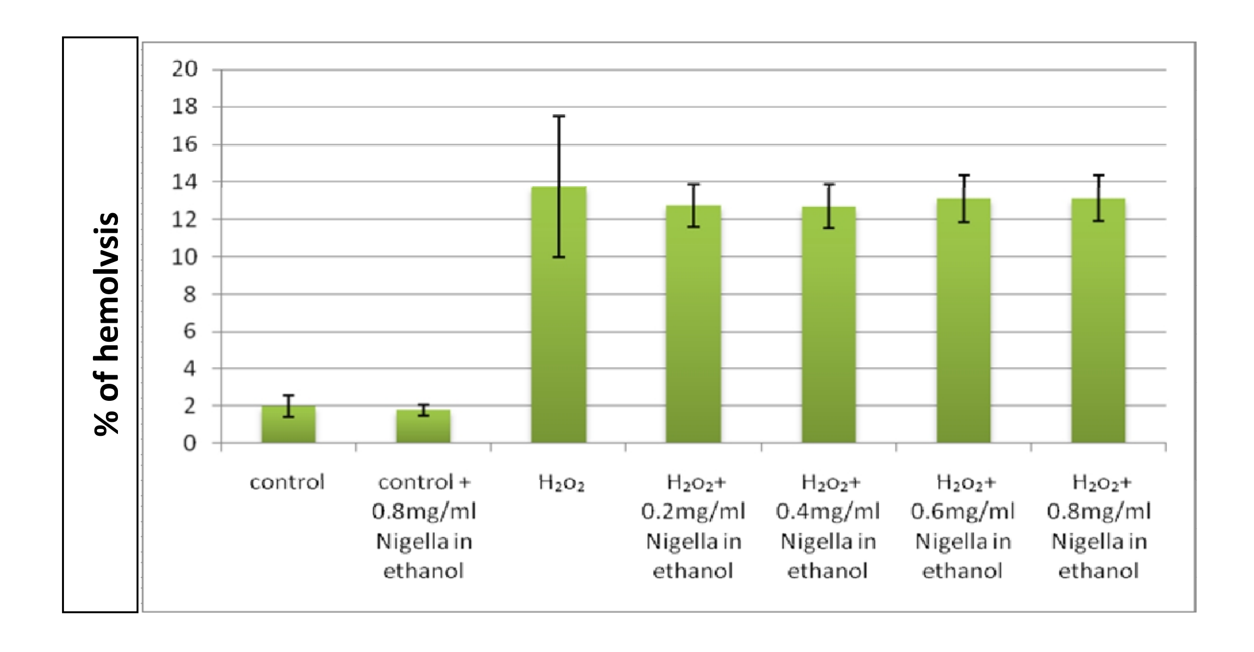


Figure 24: Percentage hemolysis of normal erythrocytes when incubated at 37°C for 60 min in the absence or in the presence of 10 mM H_2O_2, or in the presence of 10 mM H_2O_2 plus different concentrations of *Nigella sativum* ethanolic extract. Each column represents the mean value and bars represent the S.D. (n = 7).

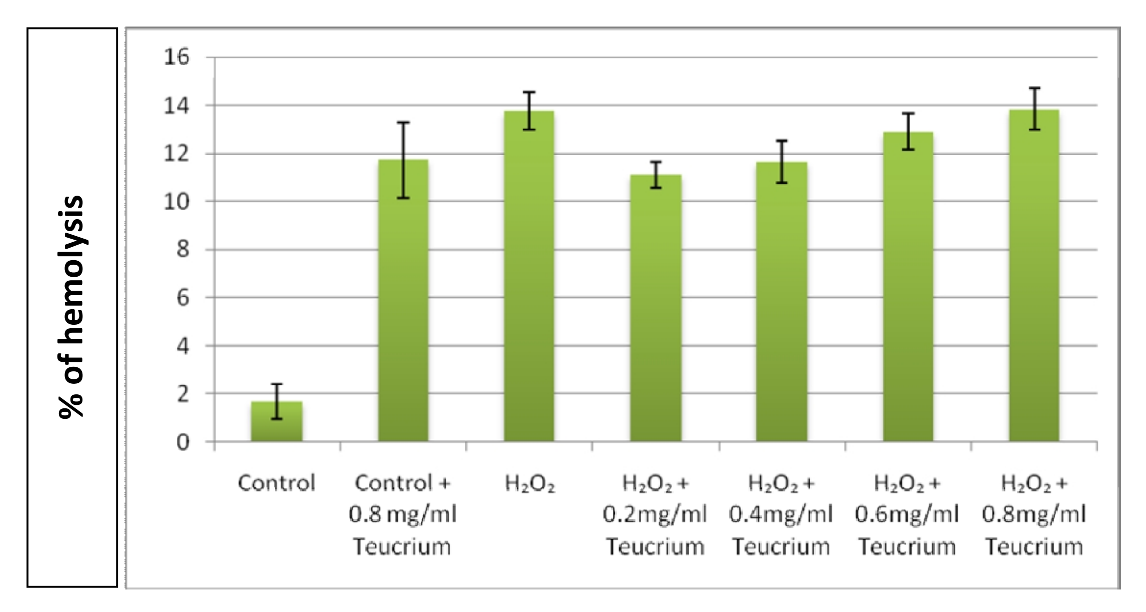


Figure 25: Percentage hemolysis of normal erythrocytes when incubated at 37°C for 60 min in the absence or in the presence of 10 mM H_2O_2, or in the presence of 10 mM H_2O_2 plus different concentrations of *Teucrium polium* methanolic extract. Each column represents the mean value and bars represent the S.D. (n = 7).

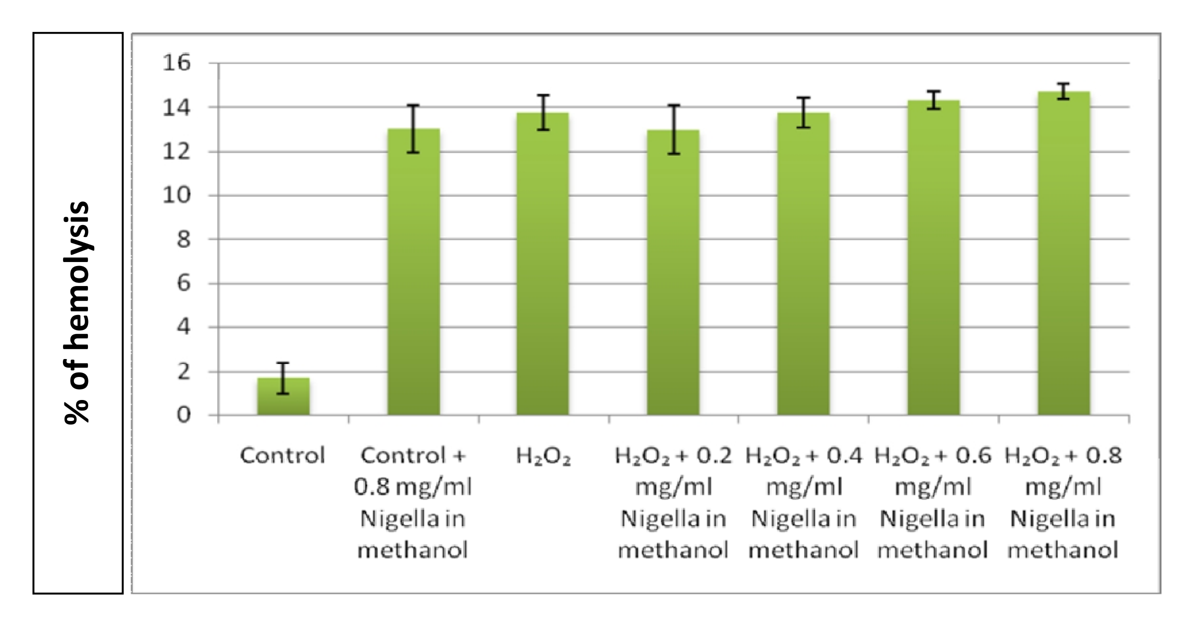


Figure 26: Percentage hemolysis of normal erythrocytes when incubated at 37°C for 60 min in the absence or in the presence of 10 mM H_2O_2, or in the presence of 10 mM H_2O_2 plus different concentrations of *Nigella sativum* methanolic extract. Each column represents the mean value and bars represent the S.D. (n = 7).

Appendix III

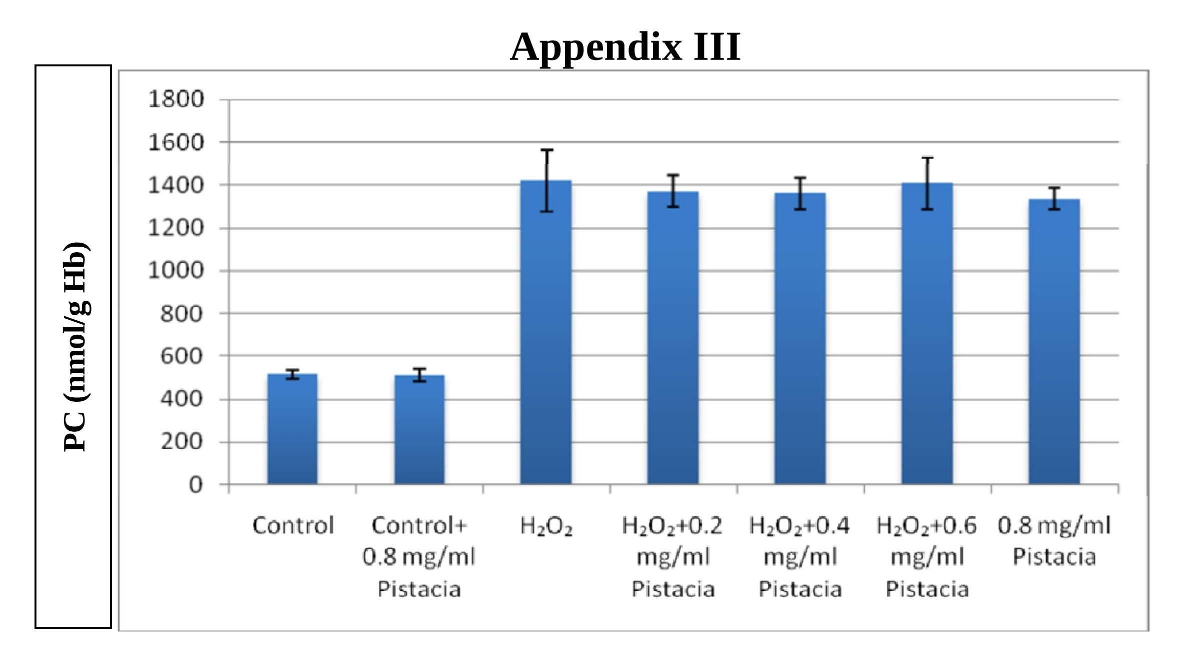


Figure 1: Protein carbonyl concentration of normal erythrocytes when incubated at 37°C for 60 min in the absence or in the presence of 10 mM H_2O_2, or in the presence of 10 mM H_2O_2 plus different concentrations of *Pistacia palaestina* methanolic extract. Each column represents the mean value and bars represent the S.D. (n = 7).

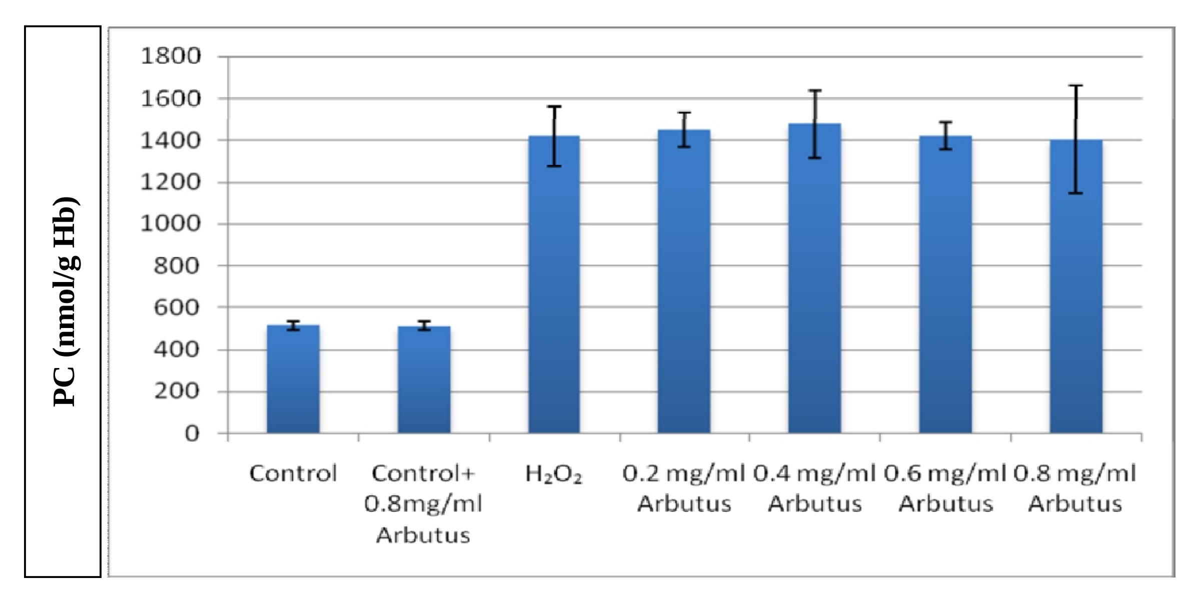


Figure 2: Protein carbonyl concentration of normal erythrocytes when incubated at 37°C for 60 min in the absence or in the presence of 10 mM H_2O_2, or in the presence of 10 mM H_2O_2 plus different concentrations of *Arbutus andrachne* methanolic extract. Each column represents the mean value and bars represent the S.D. (n = 7).

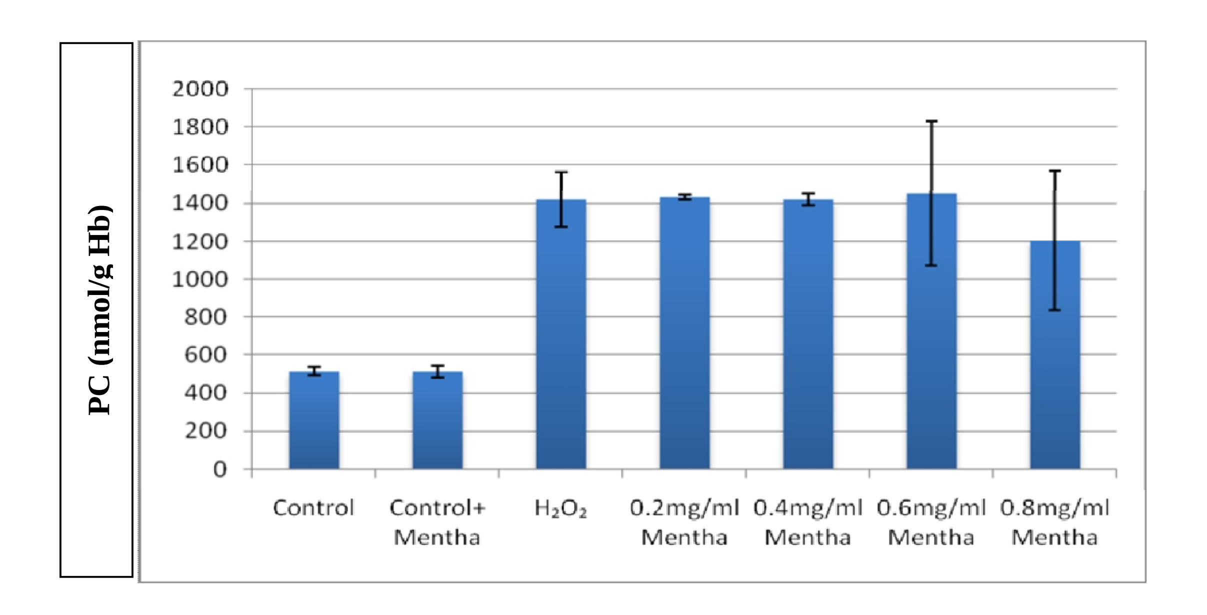


Figure 3: Protein carbonyl concentration of normal erythrocytes when incubated at 37°C for 60 min in the absence or in the presence of 10 mM H_2O_2 or in the presence of 10 mM H_2O_2 plus different concentrations of *Mentha Spp.* methanolic extract. Each column represents the mean value and bars represent the S.D. (n = 7).

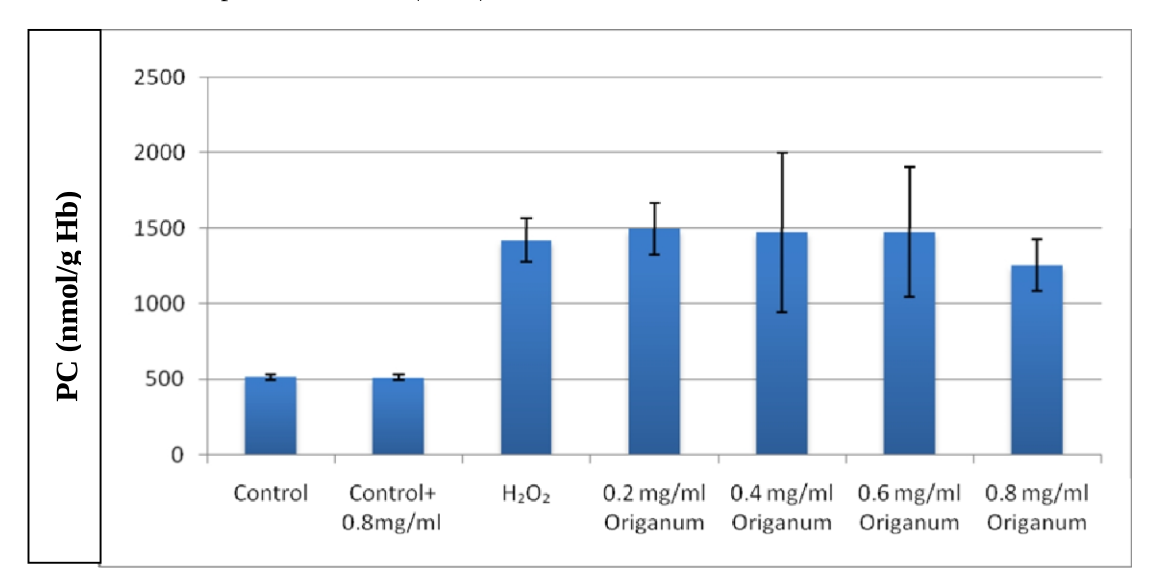


Figure 4: Protein carbonyl concentration of normal erythrocytes when incubated at 37°C for 60 min in the absence or in the presence of 10 mM H_2O_2, or in the presence of 10 mM H_2O_2 plus different concentrations of *Origanum syriacum* methanolic extract. Each column represents the mean value and bars represent the S.D. (n = 7).

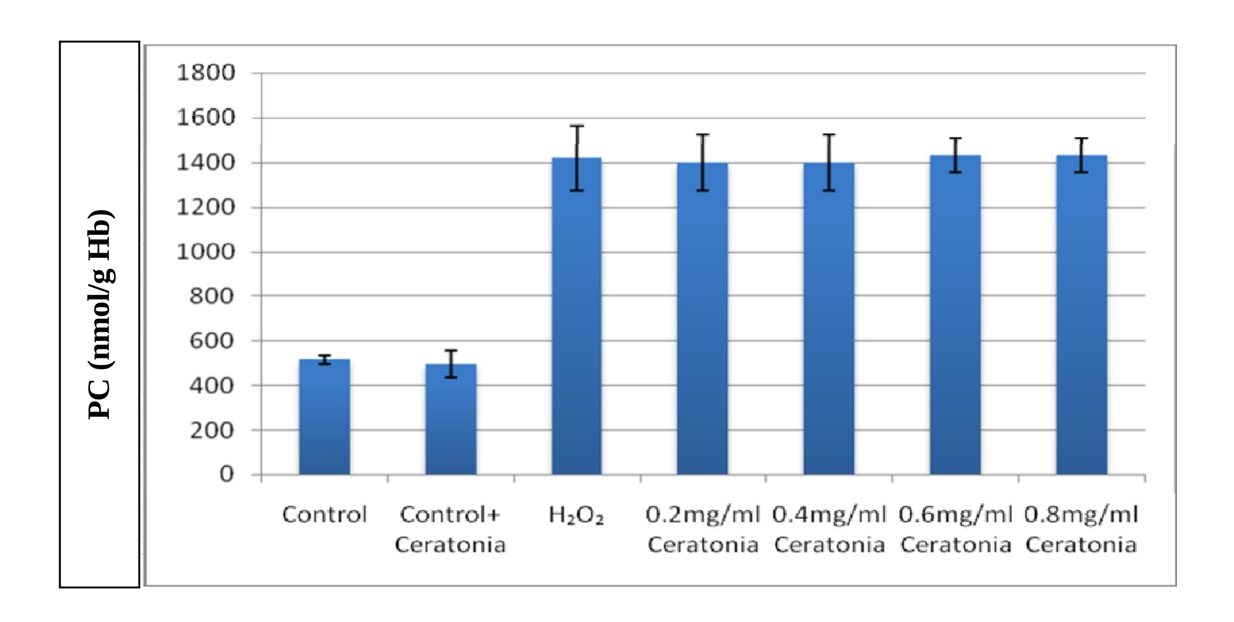


Figure 5: Protein carbonyl concentration of normal erythrocytes when incubated at 37°C for 60 min in the absence or in the presence of 10 mM H_2O_2 or in the presence of 10 mM H_2O_2 plus different concentrations of *Ceratonia siliqua* methanolic extract. Each column represents the mean value and bars represent the S.D. (n = 7).

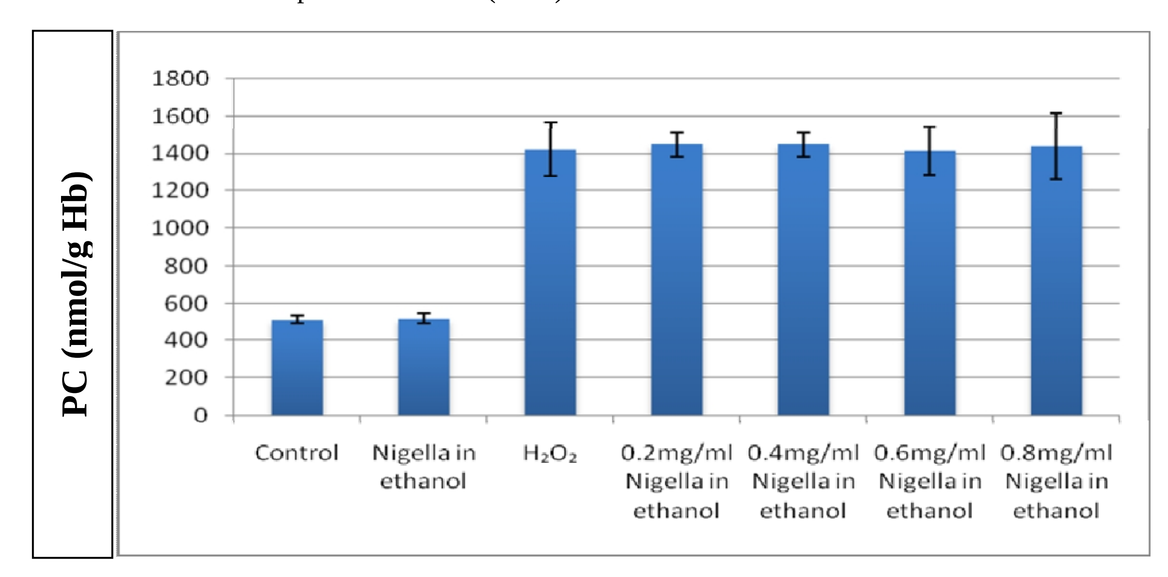


Figure 6: Protein carbonyl concentration of normal erythrocytes when incubated at 37°C for 60 min in the absence or in the presence of 10 mM H_2O_2, or in the presence of 10 mM H_2O_2 plus different concentrations of *Nigella sativum* ethanolic extract. Each column represents the mean value and bars represent the S.D. (n = 7).

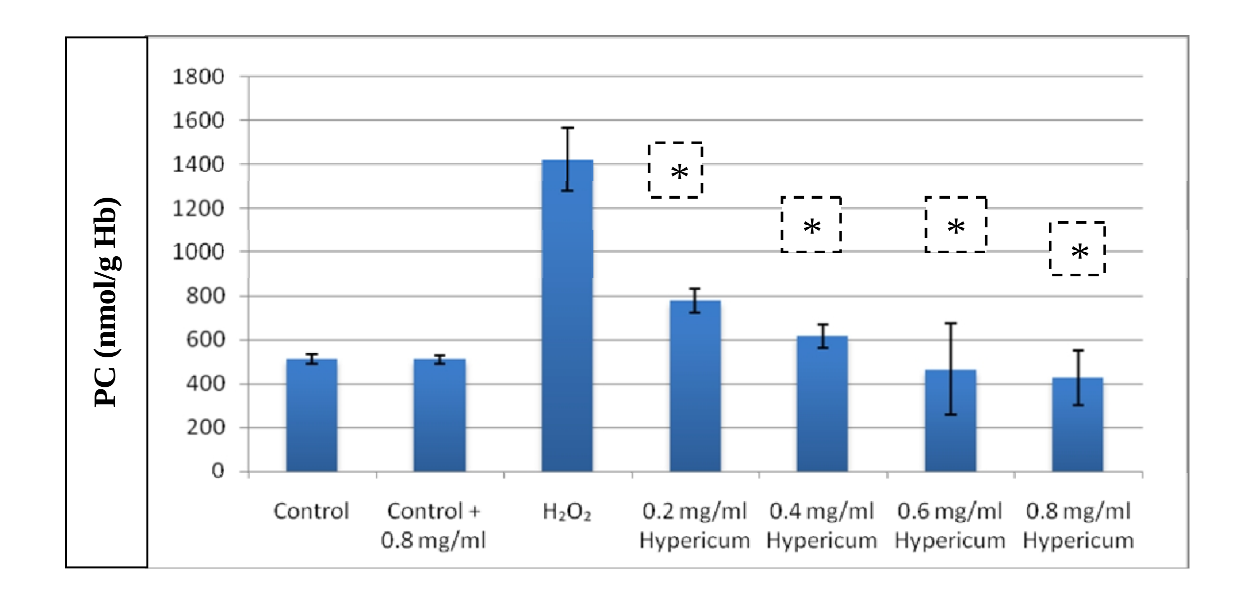


Figure 7: Protein carbonyl concentration of normal erythrocytes when incubated at 37°C for 60 min in the absence or in the presence of 10 mM H_2O_2, or in the presence of 10 mM H_2O_2 plus different concentrations of *Hypericum triquetrifolium* methanolic extract. Each column represents the mean value and bars represent the S.D. (n = 7).*P< 0.05, compared to treatment with H_2O_2 alone.

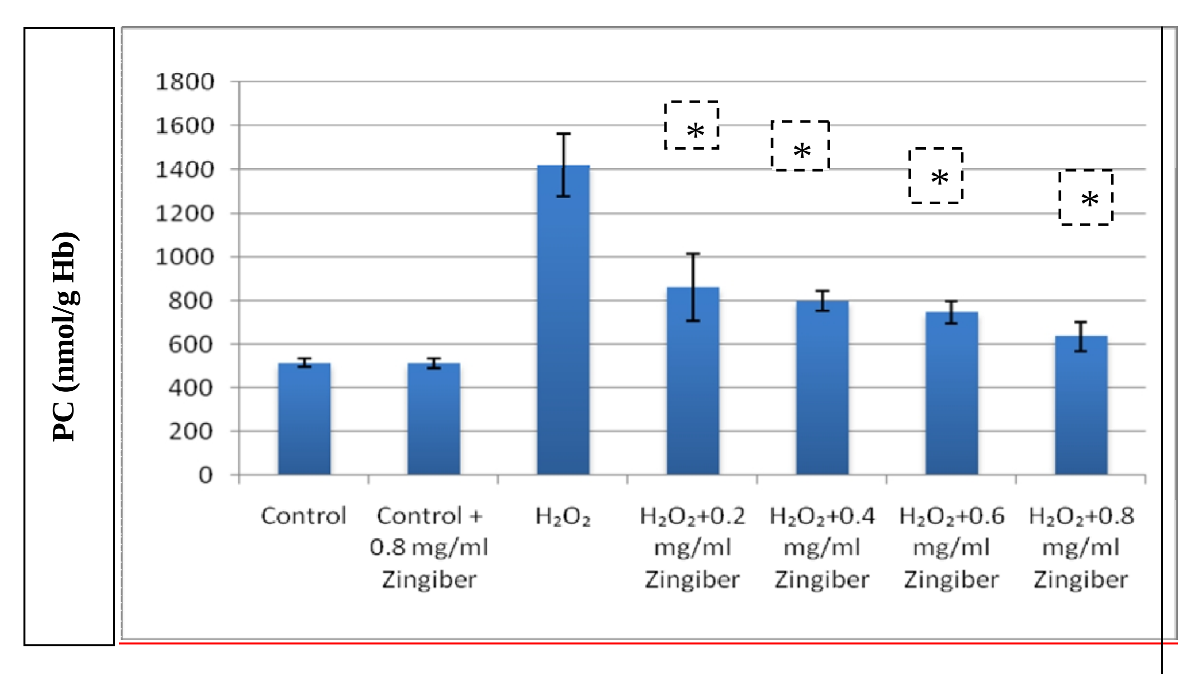


Figure 8: Protein carbonyl concentration of normal erythrocytes when incubated at 37°C for 60 min in the absence or in the presence of 10 mM H_2O_2, or in the presence of 10 mM H_2O_2 plus different concentrations of *Zingiber officinale* methanolic extract. Each column represents the mean value and bars represent the S.D. (n = 7).*P< 0.05, compared to treatment with H_2O_2 alone.

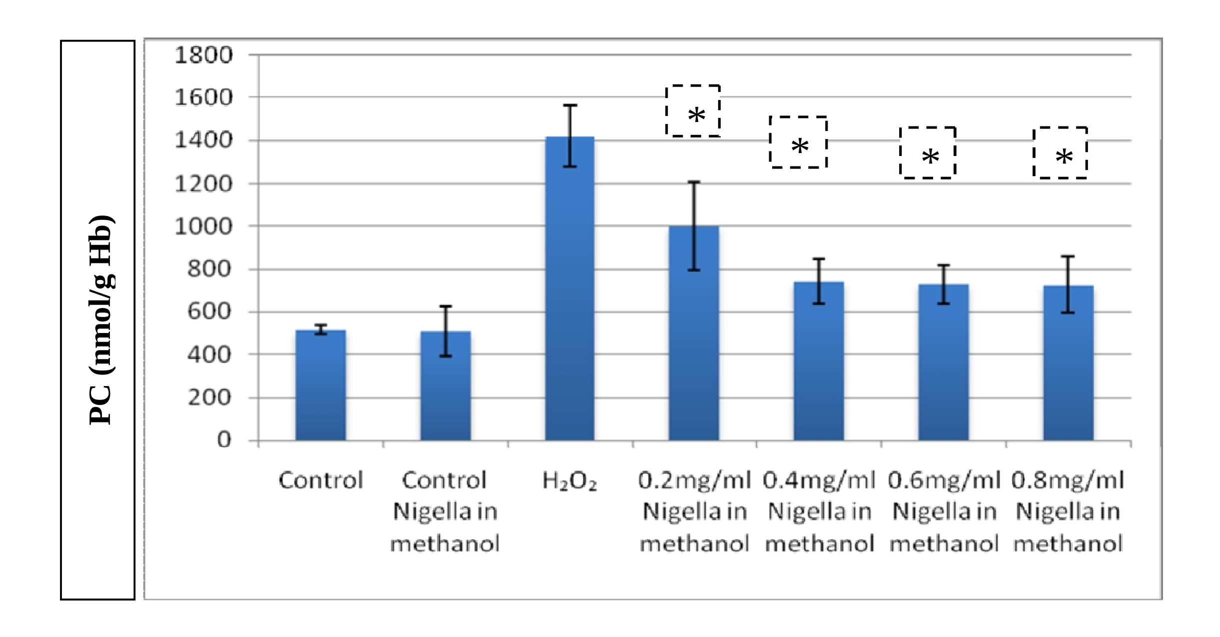


Figure 9: Protein carbonyl concentration of normal erythrocytes when incubated at 37°C for 60 min in the absence or in the presence of 10 mM H_2O_2, or in the presence of 10 mM H_2O_2 plus different concentrations of *Nigella sativum* methanolic extract. Each column represents the mean value and bars represent the S.D. (n = 7).*P< 0.05, compared to treatment with H_2O_2 alone.

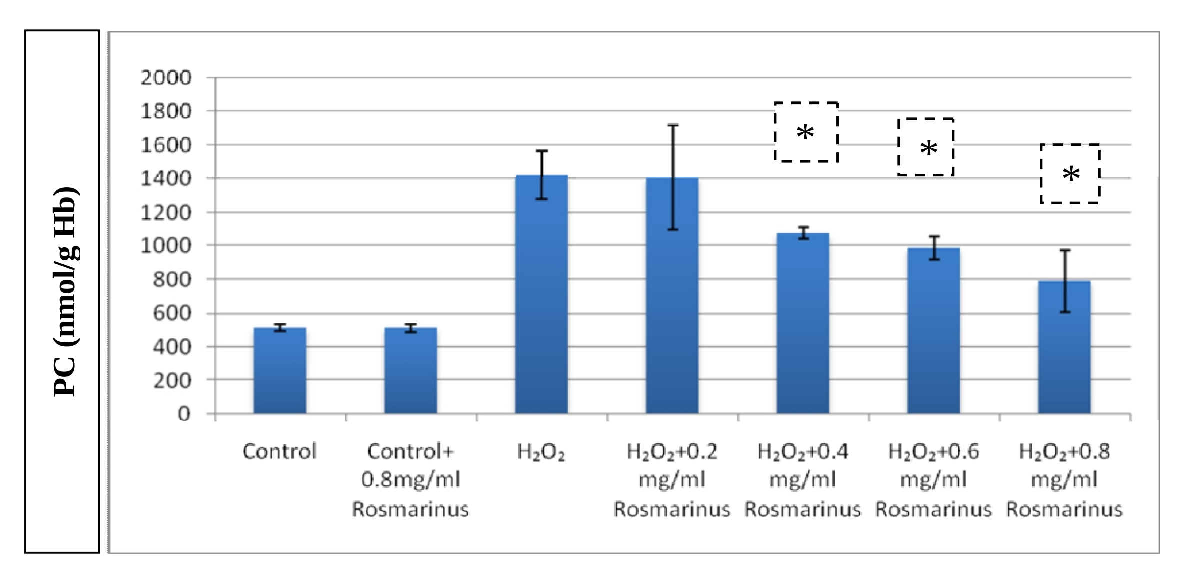


Figure 10: Protein carbonyl concentration of normal erythrocytes when incubated at 37°C for 60 min in the absence or in the presence of 10 mM H_2O_2, or in the presence of 10 mM H_2O_2 plus different concentrations of *Rosmarinus officinalis* methanolic extract. Each column represents the mean value and bars represent the S.D. (n = 7).*P< 0.05, compared to treatment with H_2O_2 alone.

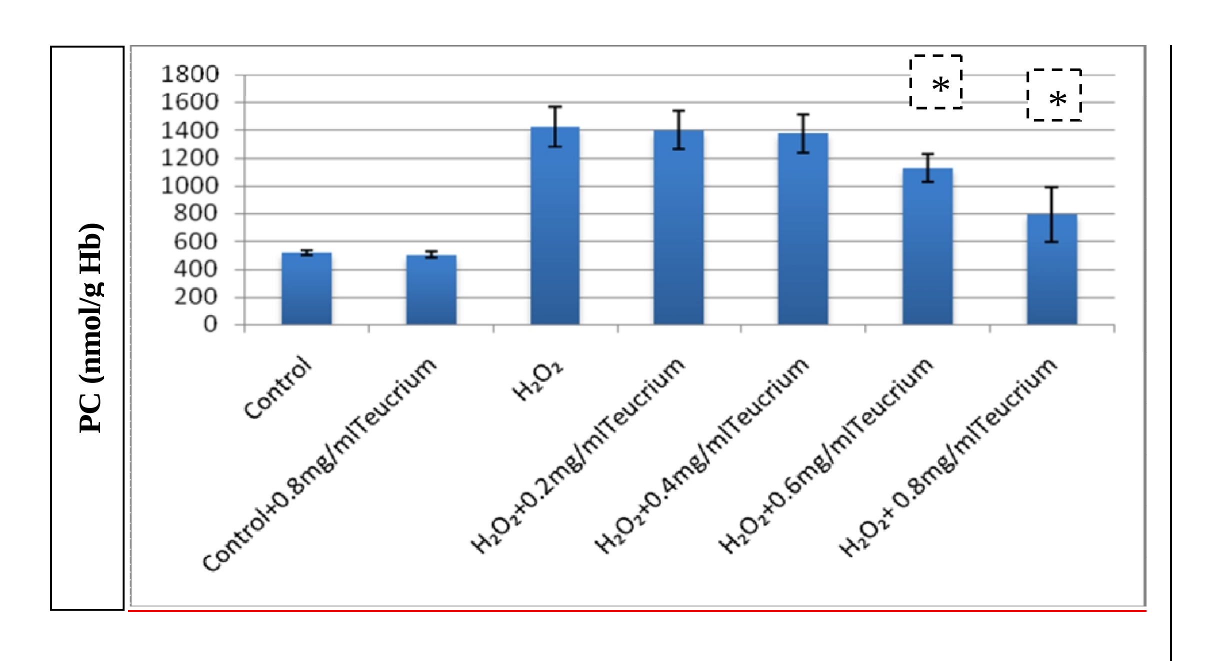


Figure 11: Protein carbonyl concentration of normal erythrocytes when incubated at 37°C for 60 min in the absence or in the presence of 10 mM H_2O_2, or in the presence of 10 mM H_2O_2 plus different concentrations of *Teucrium polium* methanolic extract. Each column represents the mean value and bars represent the S.D. (n = 7).*P< 0.05, compared to treatment with H_2O_2 alone.

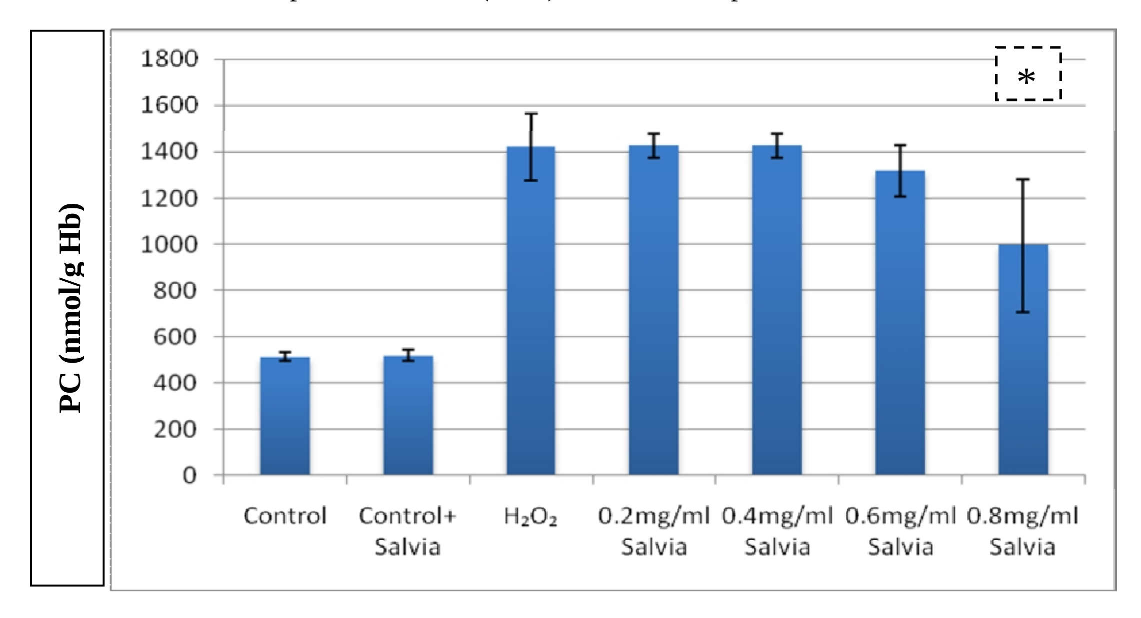


Figure 12: Protein carbonyl concentration of normal erythrocytes when incubated at 37°C for 60 min in the absence or in the presence of 10 mM H_2O_2, or in the presence of 10 mM H_2O_2 plus different concentrations of *Salvia triloba* methanolic extract. Each column represents the mean value and bars represent the S.D. (n = 7).*P< 0.05, compared to treatment with H_2O_2 alone.

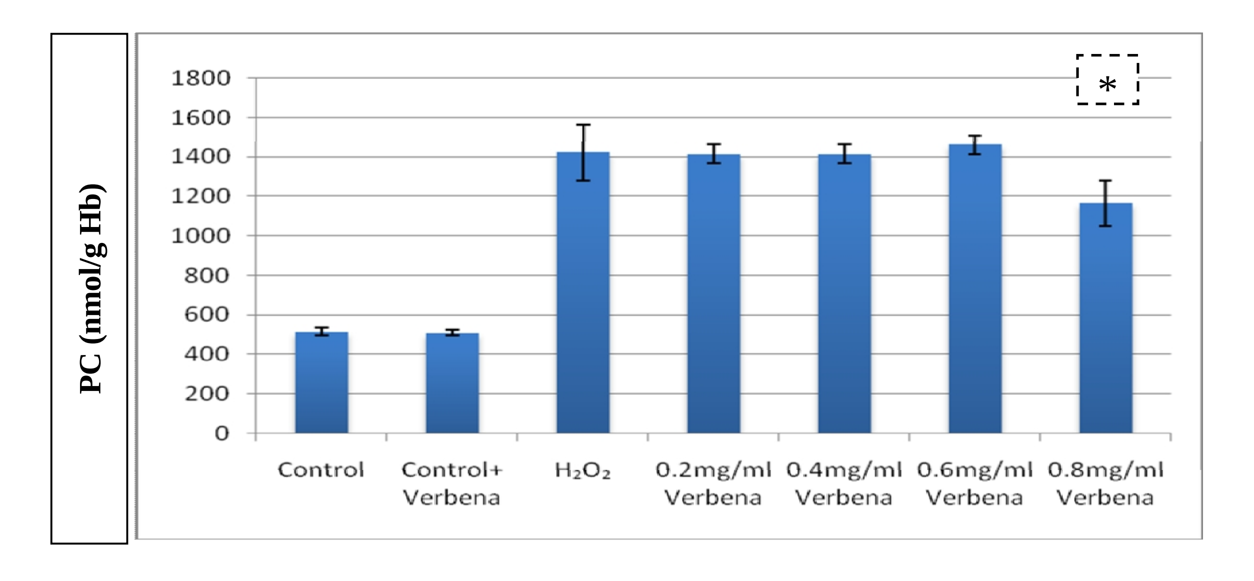


Figure 13: Protein carbonyl concentration of normal erythrocytes when incubated at 37°C for 60 min in the absence or in the presence of 10 mM H_2O_2, or in the presence of 10 mM H_2O_2 plus different concentrations of *Verbena triphylla* methanolic extract. Each column represents the mean value and bars represent the S.D. (n = 7).*P< 0.05, compared to treatment with H_2O_2 alone.

Appendix IV

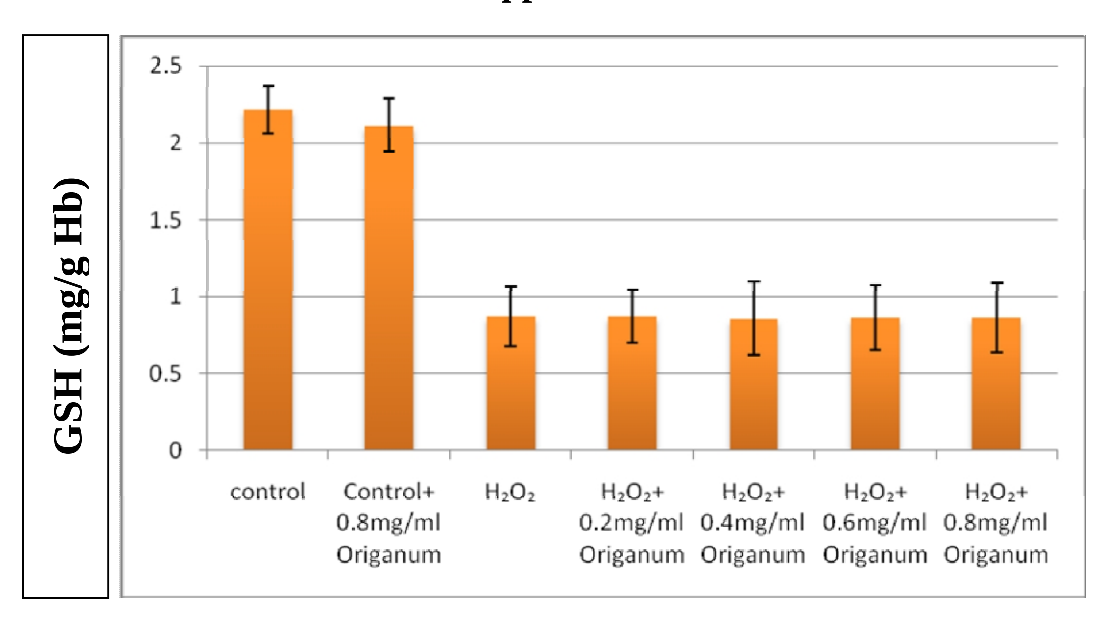


Figure 1: Reduced glutathione concentration of normal erythrocytes when incubated at 37°C for 60 min in the absence or in the presence of 10 mM H_2O_2, or in the presence of 10 mM H_2O_2 plus different concentrations of *Origanum syriacum* methanolic extract. Each column represents the mean value and bars represent the S.D. (n = 7).

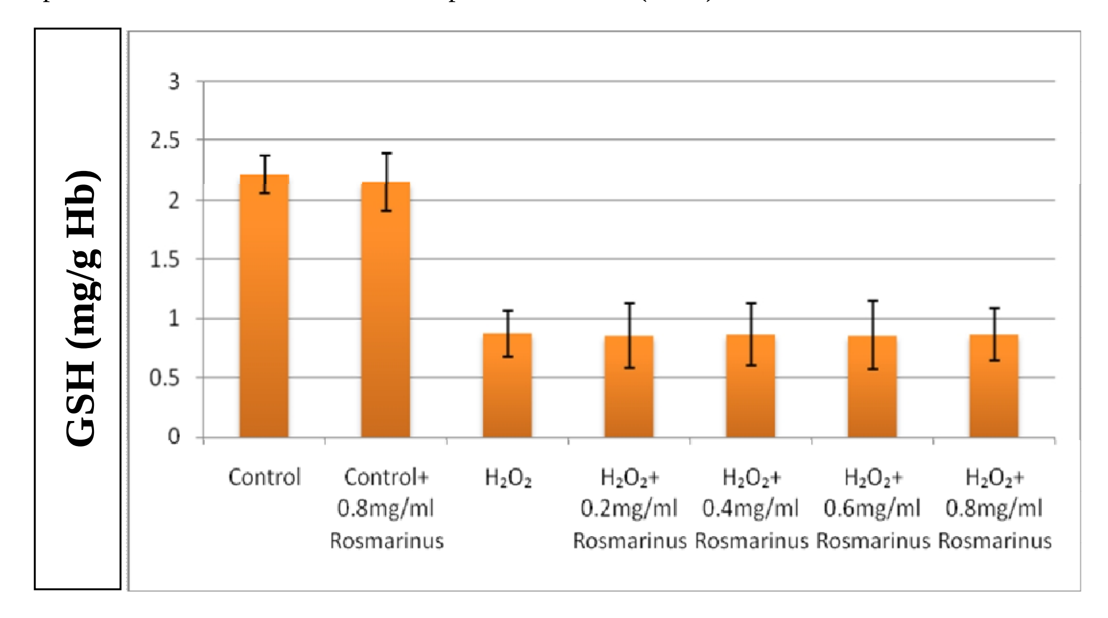


Figure 2: Reduced glutathione concentration of normal erythrocytes when incubated at 37°C for 60 min in the absence or in the presence of 10 mM H_2O_2, or in the presence of 10 mM H_2O_2 plus different concentrations of *Rosmarinus officinalis* methanolic extract. Each column represents the mean value and bars represent the S.D. (n = 7).

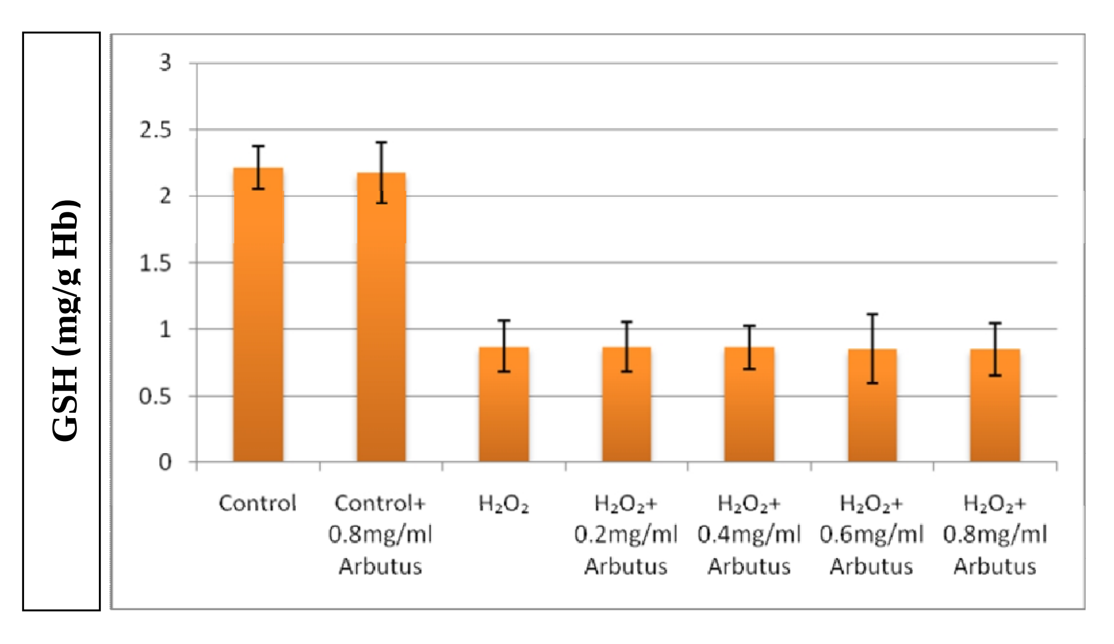


Figure 3: Reduced glutathione concentration of normal erythrocytes when incubated at 37°C for 60 min in the absence or in the presence of 10 mM H_2O_2, or in the presence of 10 mM H_2O_2 plus different concentrations of *Arbutus andrachne* methanolic extract. Each column represents the mean value and bars represent the S.D. (n = 7).

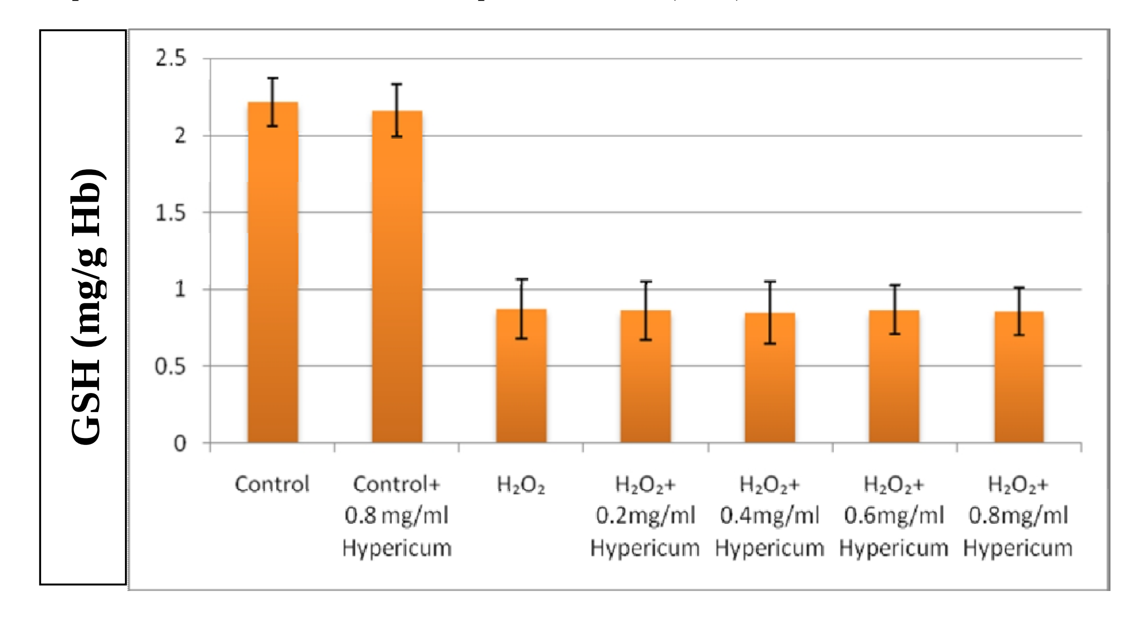


Figure 4: Reduced glutathione concentration of normal erythrocytes when incubated at 37°C for 60 min in the absence or in the presence of 10 mM H_2O_2, or in the presence of 10 mM H_2O_2 plus different concentrations of *Hypericum triquetrifolium* methanolic extract. Each column represents the mean value and bars represent the S.D. (n = 7).

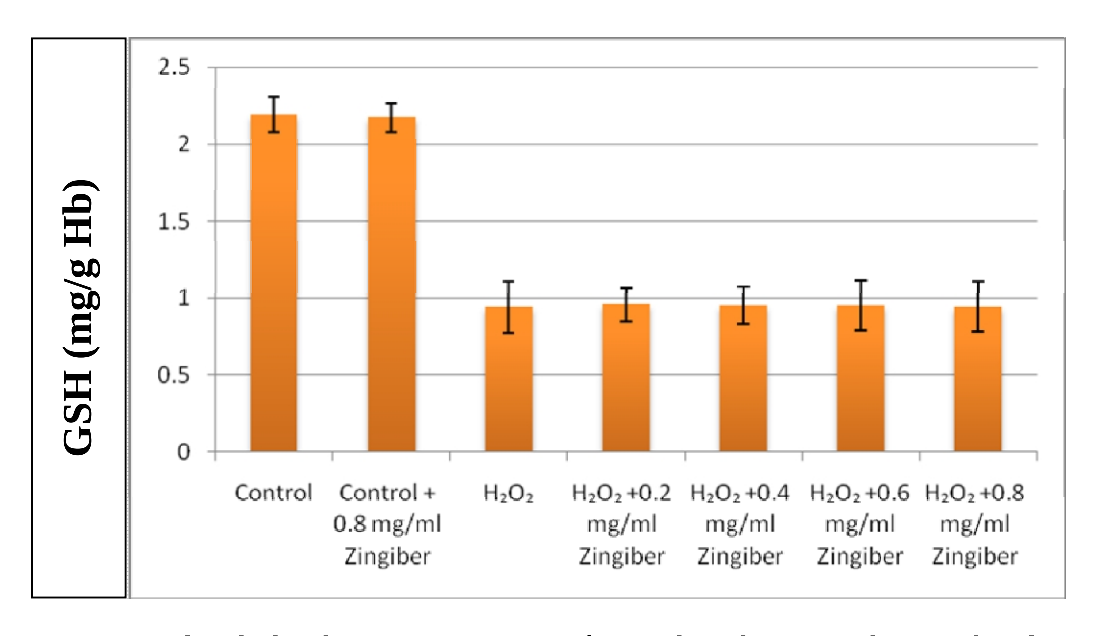


Figure 5: Reduced glutathione concentration of normal erythrocytes when incubated at 37°C for 60 min in the absence or in the presence of 10 mM H_2O_2, or in the presence of 10 mM H_2O_2 plus different concentrations of *Zingiber officinale* methanolic extract. Each column represents the mean value and bars represent the S.D. (n = 7).

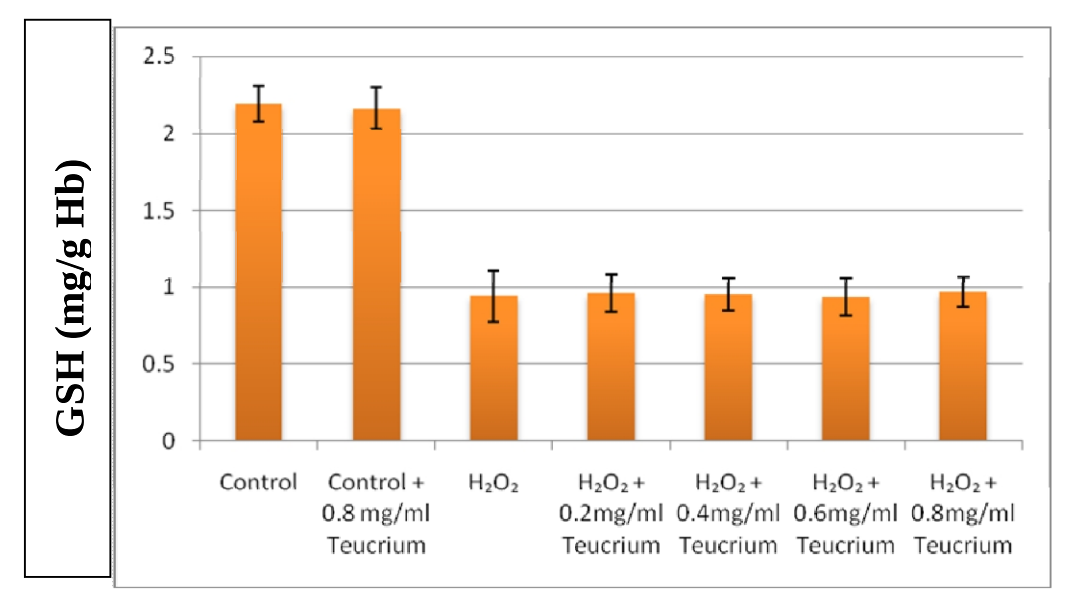


Figure 6: Reduced glutathione concentration of normal erythrocytes when incubated at 37°C for 60 min in the absence or in the presence of 10 mM H_2O_2, or in the presence of 10 mM H_2O_2 plus different concentrations of *Teucrium polium* methanolic extract. Each column represents the mean value and bars represent the S.D. (n = 7).

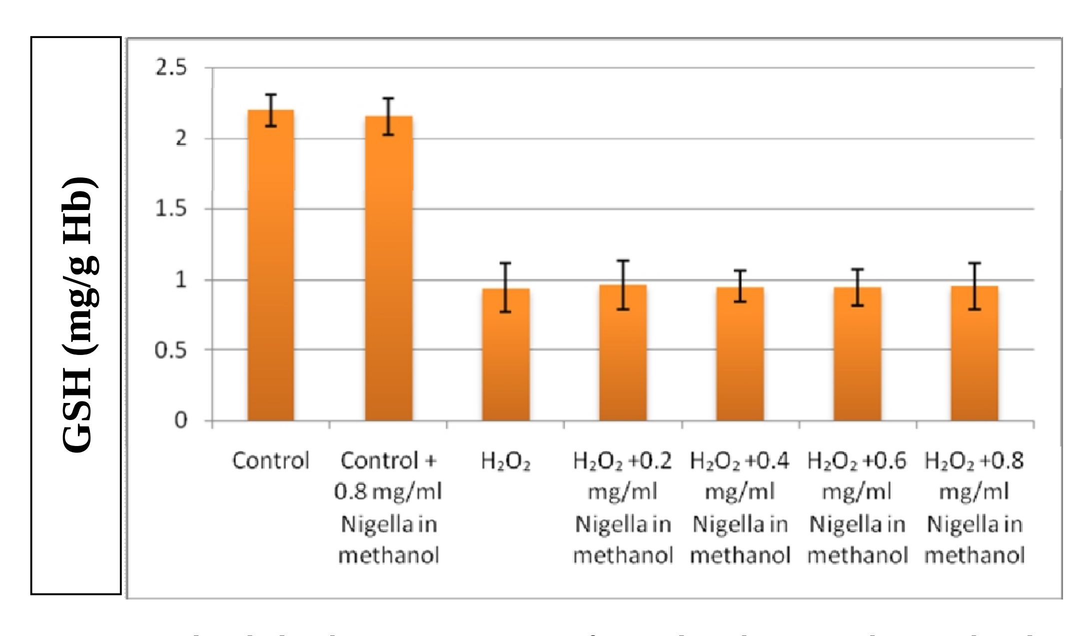


Figure 7: Reduced glutathione concentration of normal erythrocytes when incubated at 37°C for 60 min in the absence or in the presence of 10 mM H_2O_2, or in the presence of 10 mM H_2O_2 plus different concentrations of *Nigella sativum* methanolic extract. Each column represents the mean value and bars represent the S.D. (n = 7).

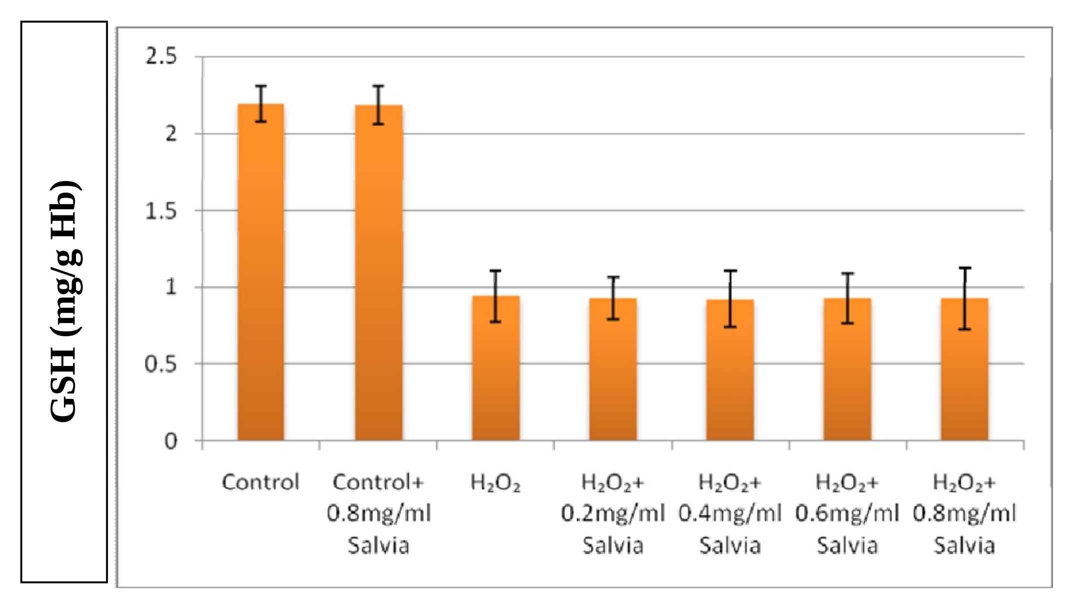


Figure 8: Reduced glutathione concentration of normal erythrocytes when incubated at 37°C for 60 min in the absence or in the presence of 10 mM H_2O_2, or in the presence of 10 mM H_2O_2 plus different concentrations of *Salvia triloba* methanolic extract. Each column represents the mean value and bars represent the S.D. (n = 7).

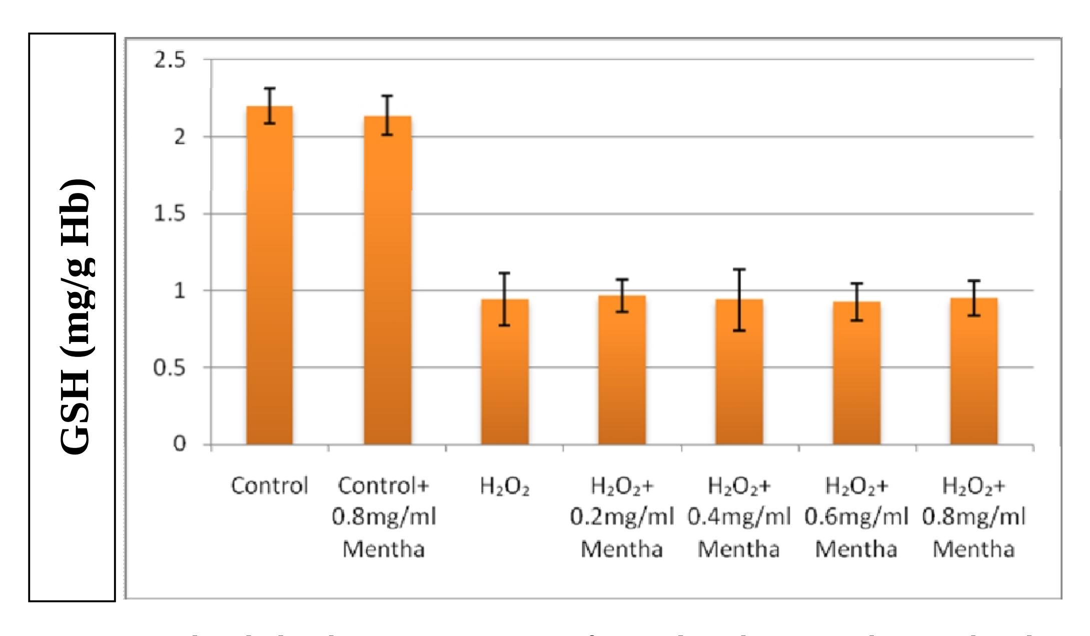


Figure 9: Reduced glutathione concentration of normal erythrocytes when incubated at 37°C for 60 min in the absence or in the presence of 10 mM H_2O_2, or in the presence of 10 mM H_2O_2 plus different concentrations of *Mentha Spp.* methanolic extract. Each column represents the mean value and bars represent the S.D. (n = 7).

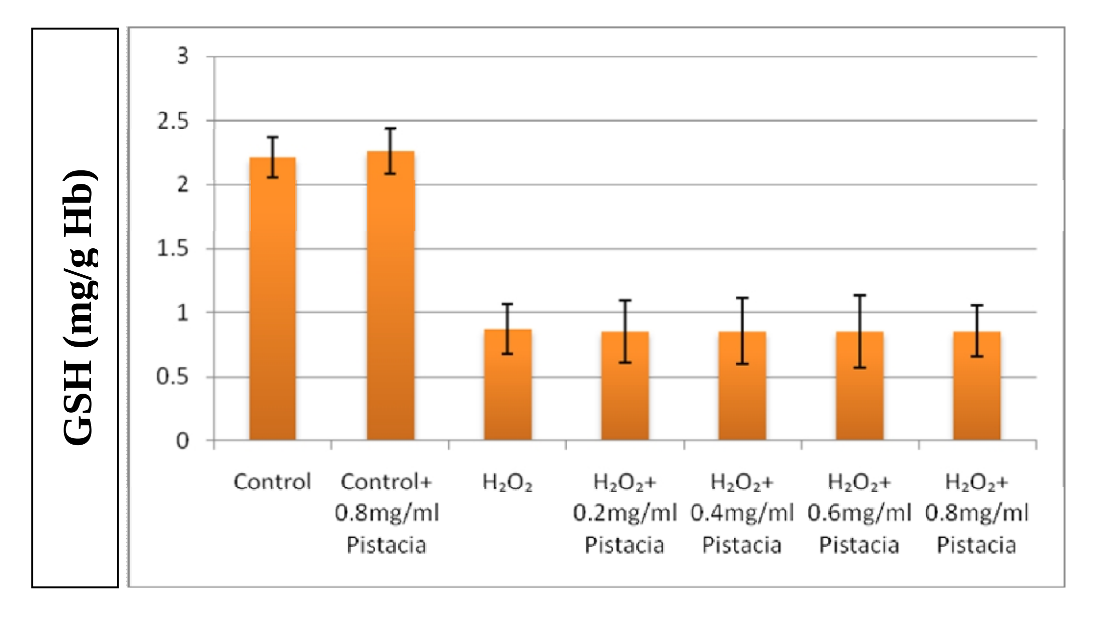


Figure 10: Reduced glutathione concentration of normal erythrocytes when incubated at 37°C for 60 min in the absence or in the presence of 10 mM H_2O_2, or in the presence of 10 mM H_2O_2 plus different concentrations of *Pistacia palaestina* methanolic extract. Each column represents the mean value and bars represent the S.D. (n = 7).

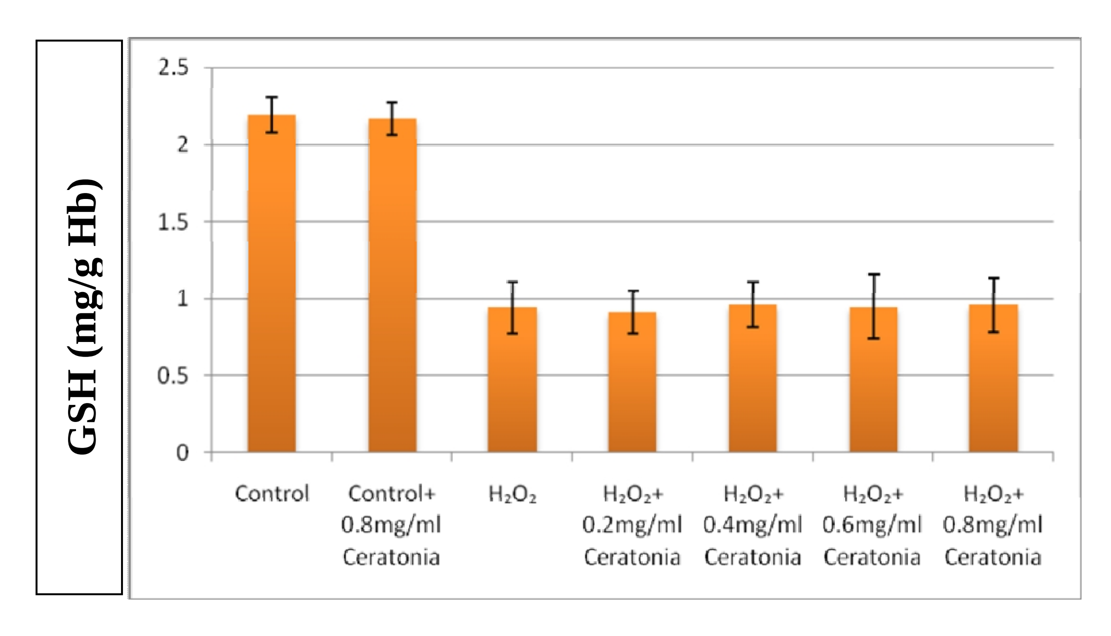


Figure 11: Reduced glutathione concentration of normal erythrocytes when incubated at 37°C for 60 min in the absence or in the presence of 10 mM H_2O_2, or in the presence of 10 mM H_2O_2 plus different concentrations of *Ceratonia siliqua* methanolic extract. Each column represents the mean value and bars represent the S.D. (n = 7).

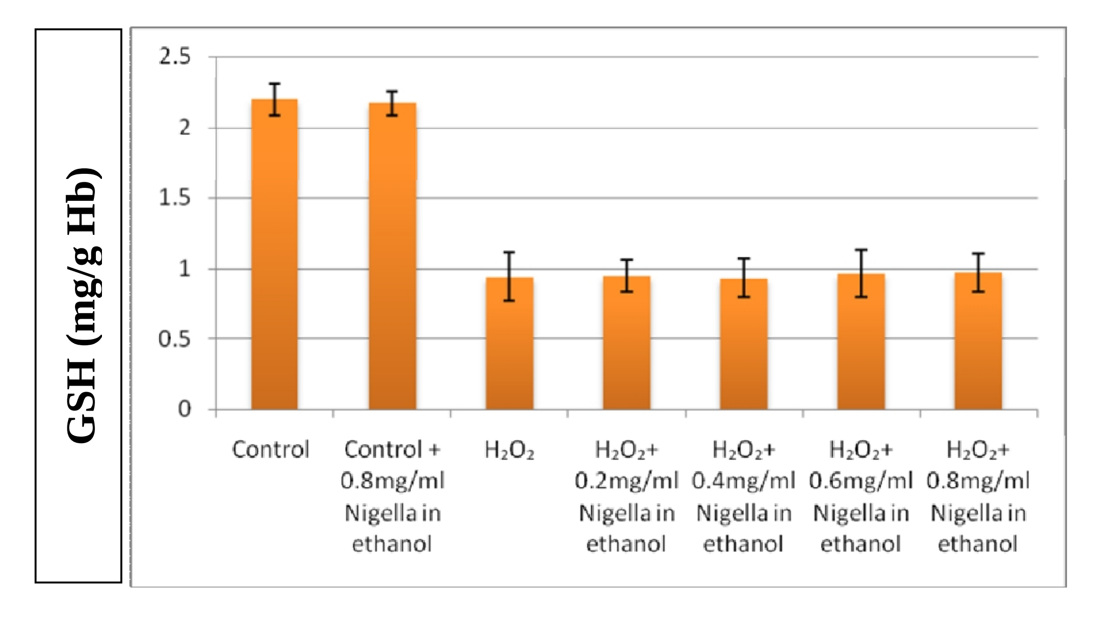


Figure 12: Reduced glutathione concentration of normal erythrocytes when incubated at 37°C for 60 min in the absence or in the presence of 10 mM H_2O_2, or in the presence of 10 mM H_2O_2 plus different concentrations of *Nigella sativum* ethanolic extract. Each column represents the mean value and bars represent the S.D. (n = 7).

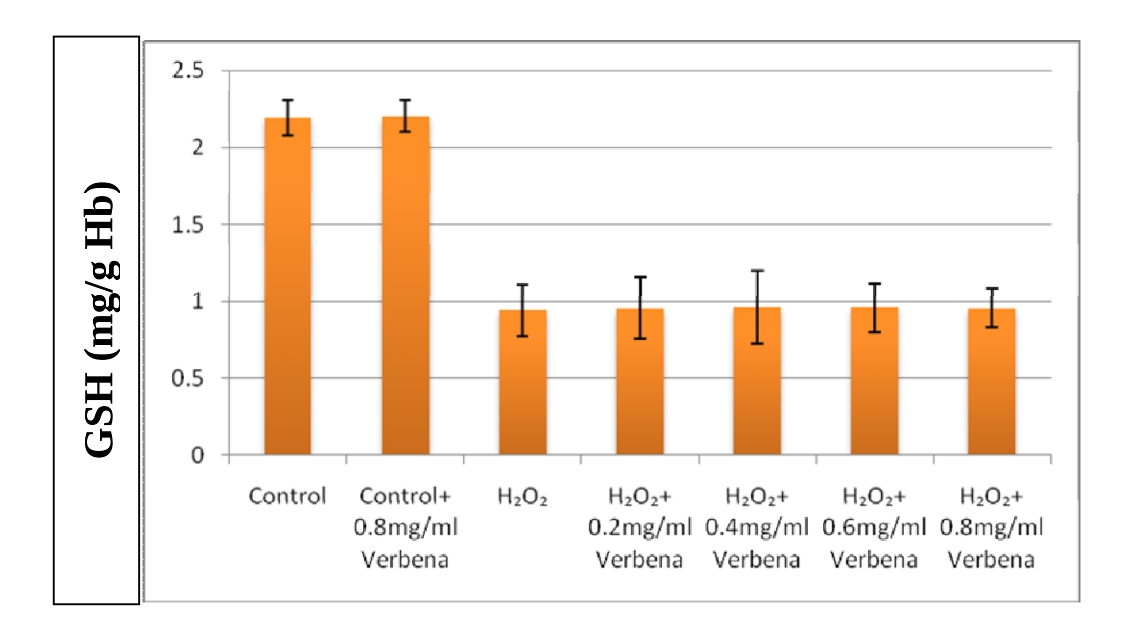


Figure 13: Reduced glutathione concentration of normal erythrocytes when incubated at 37 °C for 60 min in the absence or in the presence of 10 mM H_2O_2, or in the presence of 10 mM H_2O_2 plus different concentrations of *Verbena triphylla* methanolic extract. Each column represents the mean value and bars represent the S.D. (n = 7).

دراسات على مستخلصات من نباتات طبية مختارة كمضادات للأكسدة في دم الإنسان

إعداد

نسرين غازي العبداللات

المشرف

الاستاذ الدكتور يوسف بلتو

المشرف المشارك

الاستاذ الدكتور ماهر سليم

في الجزء الأول من هذا البحث تم دراسة خواص عدد من المستخلصات النباتية باستخدام الطرق الكيماوية التالية: القدرة الكلية المضادة للمؤكسدات، قدرة اختزال الحد يد، قدرة تحييد الجذر الحر ١,١ دايفينل بكيرل هيدرازيل، قدرة ربط الحديد، المحتوي الفينولي ومحتواها من الفلافونويدات. أعطت المستخلصات تفاوتا في الخواص المضادة للمؤكسدات وبشكل عام يمكن ترتيب المستخلصات النباتية المدروسة تنازليا حسب قدرتها المضادة للمؤكسدات محسوبة بالنسبة المئوية للمستخلص النباتي كالتالي: مستخلص ورق شجرة البطم *Pistacia palaestina* > مستخلص ورق شجرة القيقب *Arbutus andrachne* > مستخلص عشبة الروجة *Hypericum triquetrifolium* > مستخلص جدر الزنجبيل *Zingiber officinale* > مستخلص ورق النعنع *Mentha Spp.* > مستخلص ورق اكليل الجبل *Rosmarinus officinalis* > مستخلص ورق الميرمية *Salvia triloba* > مستخلص ورق المليسا *Verbena triphylla* > مستخلص ورق الزعتر *Origanum syriacum* > مستخلص ورق الجعده *Teucrium polium* > مستخلص حبة البركة *Nigella sativum* > مستخلص ثمرة الخروب *Ceratonia siliqua* . عزيت قوة الخواص المضادة للمؤكسدات للمستخلصات النباتية إلى كمية محتواها من الفينولات والفلافونويدات وكانت حبة البركة هي أقل المستخلصات نشاطا كمضاد للمؤكسدات على الرغم من أنها أقوى المستخلصات نشاطا من حيث ربط الحديد وهذا يشير إلى أن قدرتها كمضاد أكسدة في الأنظمة الحيوية يعود إلى خاصيتها على ربط أيون الحديد أكثر من خاصية تحييد الجذور الحرة، لذلك قد تكون مصدرا للأدوية المستخدمة في عزل الحديد في بعض الأمراض التي يرافقها ترسب الحديد الزائد في أنسجة الجسم المختلفة مثل مرض الثلاسيميا.

أما في الجزء الثاني من هذا البحث فقد تمت دراسة تأثيرات هذه المستخلصات النباتية على خلايا الدم الحمراء المعرضة إلى الإجهاد التأكسدي الزائد بعد تعرضها إلى فوق أكسيد الهيدروجين وذلك من خلال منعها أكسدة الدهون (من خلال قياس مالونل دي ألديهيد)، أكسدة البروتينات (من خلال قياس الكربونيل المؤكسد للبروتين) ، فقدان مستوى الجلوتاثيون المختزل في الخلية الحمراء وتحلل الخلايا الدم الحمراء نتيجة الجهد التأكسدي الزائد. إن حضن الخلايا الحمراء مع المستخلصات الميثانولية للزنجبيل ، الزعتر، إكليل الجبل، القيقب، البطم، الروجة، الميرمية، المليسا، النعنع، الجعدة، حبة البركة و الخروب سببت تقليل إنتاج مالونل دي ألديهيد في خلية الدم الحمراء المعرضة للجهد

التأكسدي الزائد مما يدل على خاصيتها كمضاد لأكسدة الدهون بينما أدت المستخلصات الميثانولية التالية: الروجة، الزنجبيل، حبة البركة، إكليل الجبل، الجعدة، الميرمية والمليسا إلى تقليل إنتاج الكربونيل المؤكسد للبروتين مما يدل على خاصيتها كمضاد لأكسدة البروتين .كما أثبتت هذه الدراسة أن المستخلصات التي أدت إلى تقليل إنتاج مالونل دي ألديهيد تقلل من نسبة تحلل خلايا الدم الحمراء الناتج عن الجهد التأكسدي الزائد وهذا يعزى إلى خاصيتها كمضادات أكسدة للدهون كسبب لمنع تحلل الخلايا الحمراء. كما أظهرت هذه الدراسة أن المستخلصات النباتية ليس لها تأثير على مستوى الجلوتاثيون المختزل لخلية الدم الحمراء.